A Comparative Risk Metric of Infection from Exposure to Pathogens in Mesophilic Anaerobic Digested (MAD) Class B Biosolids

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Alrica Lincole Joe

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DEDICATION

To the non-traditional students who decided to continue their education to earn graduate degrees, and especially to the children of underrepresented groups who aspire to be great scientists and engineers.

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"In all thy ways acknowledge Him and He shall direct thy paths." Proverbs 3:6 (KJV)

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ABSTRACT

A Comparative Risk Metric of Infection from Exposure to Pathogens in Mespohilic Anaerobic Digested (MAD) Class B Biosolids

Alrica Lincole Joe

Patrick L. Gurian, Ph.D.

Treated sewage sludge, also commonly known as biosolids, has been widely used in landscaping and farming. Biosolids enhance the soil due to their high nutrient content and capacity to hold water. However, biosolids may also contain pathogens that can threaten public health. Digestion, lime stabilization, and composting are popular treatments used to reduce the pathogenic concentration. In the United States, land application of Class B biosolids "is a routine undertaking to reuse sewage sludge" (Sen et al, 2009). Class B biosolids are treated to reduce pathogens to low levels so that they pose minimal risk to public health and the environment. There are a wide variety of pathogens potentially present in biosolids, and the hazards presented by these pathogens vary based on their initial concentration, survival time, and dose response behavior. Thus, the objective of this project was to develop a comparative metric for the risk of infection from exposure to current concentrations of pathogens in mesophilic, anaerobic digested (MAD) Class B biosolids through the ingestion pathway. Various bacteria, viruses, and parasites were selected as pathogens of interest due to their risk of causing food and waterborne illnesses like gastroenteritis and infant diarrhea (Jones and Martin, 2003). Specifically, the aims of this research were to: (1) compile the most current and accurate data on the occurrence, dose-response, and decay parameters for as many pathogens as possible; (2) prioritize and classify pathogens as high, medium, or low risk; (3) fit distributional plots to the risk metric; and (4) determine which parameter

contributed the most to the overall uncertainty. Results imply that special attention should be focused on *Giardia*, Adenovirus, *Ascaris*, Hepatitis A, and Rotavirus as they may present a high risk of infection if present in MAD Class B Biosolids. Ingestion of protozoa and viruses at infectious doses can lead to gastroenteritis, respiratory illness, heart disease, and paralysis (Metcalf and Eddy, 2003; Haas et al., 1999; and Straub et al., 1993). A number of statistical distributions were evaluated for their ability to fit the calculated values of the metrics for different pathogens. The Weibull model best fitted both the *RRMO* and *RRM* with ranges over seven and eight orders of magnitude, respectively. Results also showed that dose-response and decay parameters contributed the most uncertainty to the risk metrics developed here. Although occurrence was shown to not contribute as considerably as dose-response and decay, sufficient data to include occurrence in the assessment were available for only six pathogens.

CHAPTER 1: INTRODUCTION AND REVIEW OF LITERATURE

1.1 Statement of Problem

In the United States, land application of Class B biosolids "is a routine undertaking to reuse sewage sludge" (Sen et al., 2009). Class B biosolids undertaking consist of sludge that has been treated to reduce pathogens to low levels so that they pose minimal risk to public health and the environment. Biosolids enhance the soil due to their high nutrient content and capacity to hold water. It was projected that in 2010 approximately 8.2 million dry tons of sewage sludge would be generated in the U.S. alone (Harrison et al., 2003). However, biosolids may also contain pathogens that can threaten public health. There are a wide variety of pathogens potentially present in biosolids, and the hazards presented by these pathogens vary based on their initial concentration, dose-response behavior, and survival in the environment.

Digestion, lime stabilization, and composting are popular treatments used to reduce pathogenic concentrations. As a result of effective sewage sludge treatment that has been established by the Environmental Protection Agency (EPA) since 1993, the occurrence of pathogens in Class B Biosolids has generally decreased. "Traditional biosolids stabilization processes such as mesophilic anaerobic and aerobic digestion reduce pathogenic bacteria concentrations by 0.5 to 4 $\log_{10}$, viruses by 0.5 to 2 $\log_{10}$, and parasites by less than 0.5 $\log_{10}$ units" (Ahmed, 1997). However, due to insufficient and antiquated data, parameters to quantify risk of infection from pathogens might have extensive uncertainty (Haas, 1999). Most of the literature available on concentration, survival time, and dose response of pathogens in biosolids were published before the EPA "final rule for Standards for the Use and Disposal of Sewage Sludge" (Ahmed,

1997). Utilizing such data may not accurately portray the risk of infection from pathogens in Class B biosolids.

1.2 Purpose of Research

The focus of this research was to develop a comparative metric for the risk of infection from exposure to current concentrations of pathogens in mesophilic, anaerobic digested (MAD) Class B biosolids through the ingestion pathway. Various bacteria, viruses, and parasites were selected as pathogens of interest due to their risk of causing food and waterborne illnesses like gastroenteritis and diarrhea (Jones and Martin, 2003). Specifically, the aims of this research were as follows: (1) compile the most current and accurate data on the occurrence, dose-response, and decay parameters for as many pathogens as possible; (2) classify and prioritize pathogens as high, medium, or low risk; (3) fit distributional plots to the risk metric; and (4) determine which parameter contributed the most to the overall uncertainty.

1.3 Definition of Biosolids

Biosolids can be defined as the "solid, semisolid, or liquid" organic residues resulting from commercial, industrial, municipal wastewater treatment (Pepper et al., 2006; Department of Health and Human Services, 2002). According to the National Research Council (NRC), biosolids are treated sewage sludge that meet the land application standards of the EPA Use or Disposal of Sewage Sludge (Code of Federal Regulations (CFR) 40 Part 503 Rule) (EPA, 1993). This rule set numerical limits for pollutants, established operational standards for pathogens and reduction in odor and vector attraction, and management practices for the reuse and disposal of wastewater sludge (Metcalf and Eddy, 2003; NRC, 2002). This sludge-derived product has similar

characteristics of soil-enhancing fertilizers. Biosolids enhance the soil due to their rich nutrients like nitrogen, potassium, and phosphorous. Biosolids exhibit the capacity to hold water in soil, and help to raise the pH of soil with the addition of lime kiln dust. These biosolids are created in an effort to sanitize water and sewages to meet the regulation standards of the 1972 Clean Water Act, before these waters are released into streams and rivers. Although produced for a good cause, biosolids may become more toxic due to the increase in the degree of treatment of wastewater. It may contain contaminants such as pathogens from human and animal feces and garbage grindings commonly found in household wastewater. Biosolids may also contain toxic heavy metals, synthetic organic chemicals, as well as organic and inorganic waste from industrial and commercial facilities. Safely disposing of biosolids in a manner that protects the public's health and safety, and the environment is one of today's major issues.

1.3.1 Disposal Methods

Communities are facing an increase in sewage sludge produced annually (Harrison et al, 2002). "Each person discharging human waste to a wastewater treatment system produces approximately 47 dry pounds (21 kilograms) of sewage sludge each year" (EPA 1993). EPA (2000a) projected that by 2016, the amount of wastewater released to public owned treatment works (POTWs) will increase by 88 percent. This increase has led to intense research of possible disposal methods. Some conventional methods have been banned by law or became difficult and expensive to maintain. For example, many coastal areas used ocean dumping as the main source of disposal; however it was banned in the United States in 1988 by the Ocean Dumping Ban Act. Many sludge incinerators were

used for some time, but they began to be recognized as major air polluters. Other options are to reuse or recycle biosolids. Heat-dried biosolids are emerging as a promising option. Heat-dried biosolids have roughly the same heat value as low grade coal. Biosolids can be used as fuel for steam to generate electricity. According to Amit Mor, CEO of Israeli investing and consulting firm Eco Energy, a ton of high-quality biosolids can produce about 30 kilograms, or 66 pounds, of good quality light oil (Kanellos, 2006). Other products such as methane, acetate, ethanol, and butyrate are being currently developed by bioconversion of biosolids (CWMI, 1996). A more popular and cost effective use of biosolids is in land applications: reclamation, land filling, and agriculture. "It is estimated that approximately 5.6 million dry tons of sewage sludge are used or disposed of annually in the United States, of which approximately 60% are used for land application or public distribution" (NRC, 2002). Biosolids work well in land application due to their similar characteristics to soil-enhancing fertilizers. Land application of biosolids has been ongoing for several decades and continues to be supported by federal and state agencies due to its beneficial properties for landscaping and farming (EPA 1981, 1984, 1991). Although beneficial to agriculture, biosolids may contain potentially harmful chemical pollutants and pathogenic organism. Biosolids must be used in such a manner that balances benefits against acceptable effects on human health and the environment.

1.3.2 Effective Biosolids Treatments

Sludges from various wastewater treatments are required to be treated according to the land application standards of the 1993 U.S. EPA Part 503 Rule to produce biosolids suitable as to pose minimal threat to the public and environment. Appendix A lists the

various unit operation, processes, or treatment methods utilized to process sludge. Treatment processes tend either remove moisture from the sludge or "stabilize the organic material" in sludge (Metcalf and Eddy, 2003). Based on the processes used, a variety of solids are generated. Types of solids produced are screenings, grit, scum/grease, raw sludge, primary sludge, chemically precipitated sludge, activated sludge, trickling-filter sludge, digested biosolids, and composted solids (Metcalf and Eddy, 2003). Bitton et al. (1980) stated that toxic chemicals are not commonly found in biosolids. This is generally due to the physical and chemical pre-treatments processes required for wastewater. Figure 1.1 and Figure 1.2 show a schematic of the general process of treating wastewater and sludge. "However, pathogens that enter wastewater from infected individuals cannot be controlled at the source and are often concentrated in wastewater solids because of their density or through adsorption during wastewater treatment" (Ponugati, 1997).

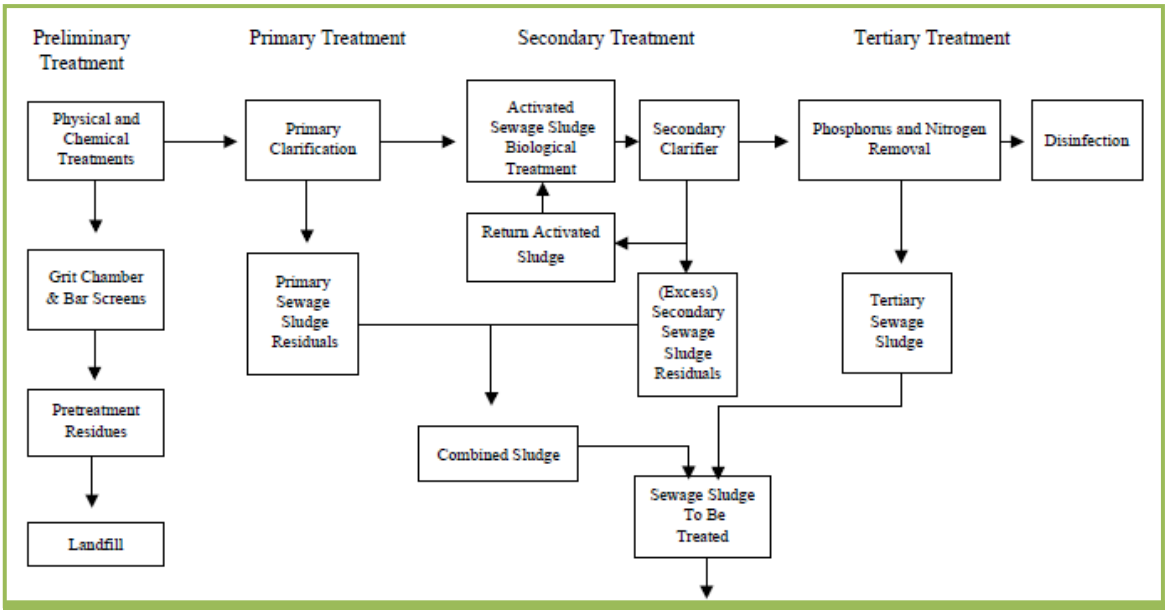

Figure 1.1 **Wastewater Treatment Processes**. The process schematic delineating water and wastewater treatment along with the sewage sludge stream. (NRC, 2002)

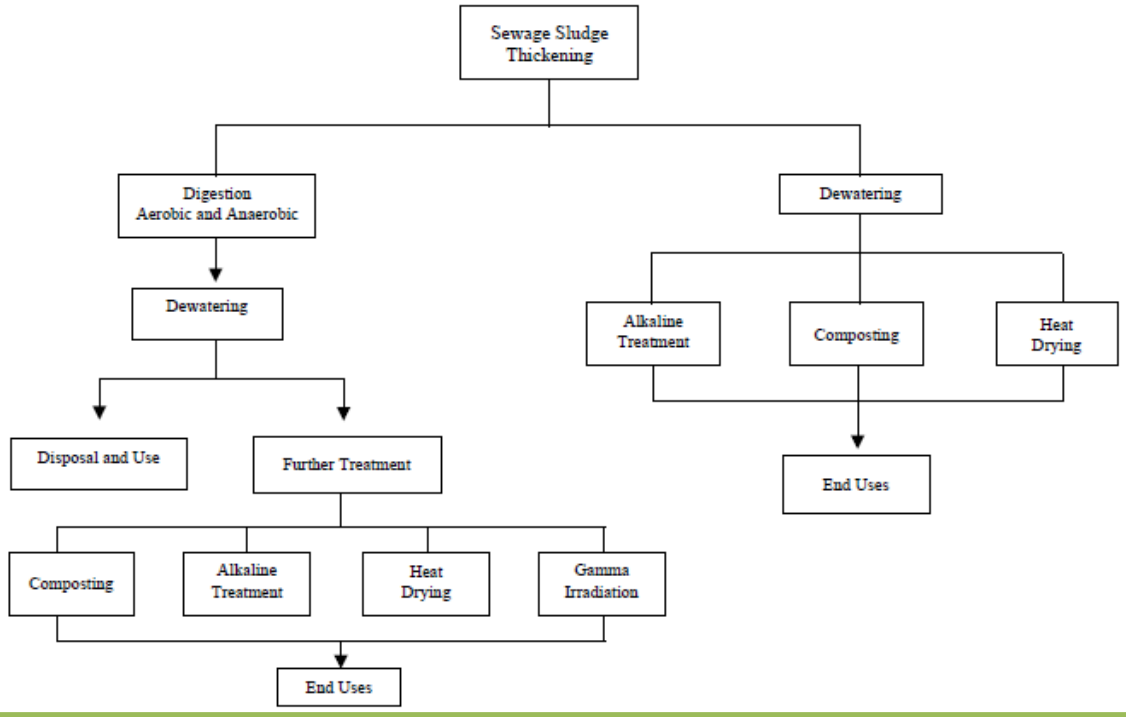

Figure 1.2 **Sewage Sludge Treatment Alternatives**. (NRC, 2002)

Reduction of pathogens absorbed in sludge can be accomplished by physical, chemical, and biological processes. The following are required treatments in order to meet the EPA standards of land applied biosolids: (1) Processes to Significantly Reduce Pathogens (PSRP) and (2) Processes to Further Reduce Pathogens (PFRP). Tables 1.1 and 1.2 give brief descriptions of these processes. PSRP reduce but do not eliminate pathogens, whereas PFRP reduce pathogens to levels below detection. PSRP are regarded as stabilization processes that also reduce volatile or organic solids, unpleasant odor, and attraction to insects and rodents. Biosolids treated by PSRP may still have the ability to spread infection (Metcalf and Eddy, 2003; NRC, 2002). In addition, "stabilization is used for volume reduction, production of useable gas (methane), and improving the

dewaterability of sludge" (Metcalf and Eddy, 2003). PFRP are considered to be disinfection processes that enhance the results of stabilization (Lucero-Ramirez, 2000).

Table 1.1 **Processes to Significantly Reduce Pathogens (PSRP).**[a]	
Process	***Description***
Facultative lagoons and storage	Animal waste and manure is treated or stored in a lagoon system at a temperature of ≤5°C (≤34°F) for a period of at least 6 mo or at a temperature of >5°C (34°F) for a period of at least 4 mo. Because all wastes must be in a lagoon for the specified period, two lagoons probably will be needed so that while one is filling, the other can be aging. This avoids short-circuiting.
Air-drying	Animal waste and manure is dried on sand beds or on paved or unpaved basins. The animal waste and manure dries for a minimum of 3 mo. During two of the three months, the ambient average daily temperature is above 0°C (32°F).
Composting	Using either the within-vessel, static aerated pile, or windrow composting methods, the temperature of the animal waste and manure is raised to 40°C (104°F) or higher and remains at 40°C (104°F) or higher for 5 d. For 4 h during the 5-d period, the temperature in the compost pile exceeds 55°C (131°F).
Anaerobic digestion	Animal waste and manure is treated in the absence of air for a specific mean cell residence time (i.e., solids retention time) at a specific temperature. Values for the mean cell residence time and temperature shall be between 15 d at 35°C to 55°C (95 to 131°F) and 60 d at 20°C (68°F).
Aerobic digestion	Animal waste and manure is agitated with air or oxygen to maintain aerobic conditions for a specific mean cell residence time (i.e., solids retention time) at a specific temperature. Values for the mean cell residence time and temperature shall be between 40 d at 20°C (68°F) and 60 d at 15°C (59°F).
Lime stabilization	Sufficient lime is added to the animal waste and manure to raise the pH of the animal wastes and manure to 12 for ≥2 h of contact.

[a]EPA, 1993

Table 1.2. **Processes to Further Reduce Pathogens (PFRP).**[a]	
Process	***Description***
Composting	Using within-vessel or static aerated pile composting, the temperature of the biosolids is maintained at 55°C (131°F) or higher for 3 d. Using windrow composing, the temperature of the wastewater sludge is maintained at 55°C (131°F) or higher for 15 d or longer. During this period, a minimum of five windrow turnings is required.
Heat-drying	Dewatered biosolids are dried by direct or indirect contact with hot gases to reduce the moisture content to 10 percent or lower. Either the temperature of solids particles exceed 80°C (176°F) or the wet-bulb temperature of the gas stream in contact with the biosolids as the biosolids leave the dryer exceeds 80°C (176°F).
Heat Treatment	Liquid biosolids are heated to a temperature of 180°C (356°F) or higher for 30 min.
Thermophilic Aerobic Digestion	Liquid biosolids are agitated with air or oxygen to maintain aerobic conditions, and the MCRT is 10 d at 55 to 60°C (131 to 140°F).
Beta-ray Irradiation	Biosolids are irradiated with beta rays from an accelerator at dosages of at least 1.0 megarad (Mrad) at room temperature (approximately 20°C (68°F)).
Gamma-ray Irradiation	Biosolids are irradiated with gamma rays from certain isotopes such as 60-cobalt or 135-cesium at dosages of at least 1.0 Mrad at room temperature (approximately 20°C (68°F)).
Pasteurization	The temperature range of the biosolids is maintained at 70°C (158°C) or higher for at least 30 min.

[a]EPA, 1993

Of these processes, anaerobic digestion is the most widely and oldest process used to treat sludge. Anaerobic digestion, through chemical and biological reactions, allows bacterial growth to occur in the absence of air. This hydrolyzes organic materials, reduces volume of the biosolids, and destroys disease-causing pathogens. One reaction, known as acidogenesis, is the fermentation of soluble organic compounds by acid-producing facultative bacteria to produces carbon dioxide and some hydrogen gas (Lucero-Ramirez, 2000; Metcalf and Eddy, 2003). Methanogenesis, another reaction, converts organic acids produced by anaerobic bacteria to mostly methane gas (Haug et al., 1998; Metcalf and Eddy, 2003). The effectiveness of an anaerobic digestion process depends on the following factors: (1) retention time, (3) temperature, (4) pH, (5) alkalinity concentrations, (6) availability of nutrients and trace metals for biological growth, and (7) inhibitory substances (Lucero-Ramirez, 2000; Metcalf and Eddy, 2003). Although each factor contributes to the overall success of anaerobic digestion, retention time and temperature are key aspects. Adequate retention time is required to ensure efficient growth of bacteria and digestion of the sludge. It the minimum retention time is not met, digestion will not proceed (WEF, 1998). Temperature facilitates metabolic activity of microorganisms, gas transfer rates, and the settling of biological solids (Metcalf and Eddy, 2003). Temperature influences the rate of digestion which establishes the minimum retention time. Anaerobic digestion performs better in the thermophilic temperature range, between 50 and 57°C (120 and 135°F); however, “most anaerobic digestion systems are designed to operate in the mesophilic temperature range, between 30 and 38°C (85 and 100°F)” (Metcalf and Eddy, 2003). The mesophilic range is more common because it requires less energy to heat and provides more stability. The

final product of biosolids can be fashioned into cake, granular, pellet or liquid form. Based on the degree of treatment utilized, two classes of biosolids are produced: Class A and Class B biosolids.

1.3.3 Class A versus Class B Biosolids

Class A and Class B biosolids are defined by the extent of treatment for pathogens including bacteria, enteric viruses, protozoa, and viable helminth ova. Due to the difficulty of separating and identifying pathogens found in biosolids, indicator microorganisms can be used to detect the presence of pathogens. Common indicator organisms that have been used are (1) total coliform bacteria, (2) fecal coliform bacteria, *Escherichia coli* (E. coli), bacteroides, fecal streptococci, enterococci, *Clostridium perfringens*, and coliphage. Class A biosolids require a high level of treatment to reduce pathogen concentrations below detection levels. The EPA 40 CFR Part 503 requires that the following be met: (1) fecal coliform density to be less than 1000 most probable number (MPN)/g of total solids (TS) , (2) *Salmonella* species (sp.) density to less than 3 MPN/4 g of TS, (3) enteric viruses to less than 1 plaque-forming unit (PFU)/4 g of TS, and (4) viable helminth ova to less than 1 per 4 g of TS (EPA, 2000). Biosolids that meet Class A pathogen requirements are safe to use on public and accessible land, such as golf courses and parks. Class A biosolids that meet other regulations such as vector attraction reduction standards and heavy metal pollutant limits can be used as fertilizers or soil amendments without site restrictions. They may be bagged and sold to individuals for lawn and garden care. PFRPs that were previously mentioned are alternative treatments that may be used to reduce pathogens below detection levels. Although pathogens levels are below detection, Class A biosolids are monitored and recorded as frequently

necessary. In contrast, Class B biosolids receive lower levels of treatment and contain concentration of pathogens that pose minimal risk to public health and the environment. The EPA 40 CFR Part 503 requires fecal coliform densities of less than 2.0×10^6 MPN/g TS to be considered Class B biosolids. Class B Biosolids are commonly generated by mesophilic anaerobic digestion (MAD) in the United States (Viau et al., 2009). These biosolids must be used in accordance with site restrictions until pathogens are reduced further by environmental factors, such as sunlight and air. Class B biosolids should not be sold to the public and can only be used in bulk on approved land sites such as agricultural lands, forests, and mine reclamation sites (NRC, 2002; Pepper et al., 2006). Biosolids that meet neither Class A nor Class B may also be used in landfilling and incineration if they meet the applicable requirements. Although biosolids are mandated to be treated to standards so as to not pose a threat to the public, there have been reports of illness after exposure to field applied digested sludge. Approximately 39 illnesses were reported from more than 328 residents; however, a causal connection has not been substantiated (Harrison et al., 2002). Biosolids should be used in such a manner that balances against acceptable effects on human health and the environment.

1.4 Pathogens of Concern

Effective treatment of wastewater may concentrate pathogens in sludge. Table 2.1 lists some of the pathogens that have been commonly found in Class B biosolids. Such organisms are of interest due to their risk of causing food and water-borne illnesses like gastroenteritis and infant diarrhea (Jones and Martin, 2003). These pathogens generally enter the environment from the runoff of land-applied livestock manure or the spread of feces of infected animals and humans (Gerba et al, 2005). This list is non-exhaustive as it

is constantly changing. Microorganisms continue to evolve and mutate, while new technologies in the detection of pathogens are becoming more advanced. "Thus, no assessment of risks associated with the land application of sewage sludge can ever be considered complete when dealing with microorganisms" (Ponugoti et al., 1997).

Table 1.3 **Some Pathogens Commonly Found in Class B Biosolids**.[a]

Bacteria	***Protozoa***	***Enteric viruses***	***Helminth Ova***
Salmonella sp.	*Crytosporidium*	Hepatitis A virus	*Ascaris lumbricoides*
Shigella sp.	*Entamoeba histolytica*	Adenovirus	*Ascaris suum*
Yersinia	*Giardia lamblia*	Norovirus	*Trichuris trichirua*
Vibrio cholera	*Balantidium coli*	Sapporovirus	*Toxocara canis*
Campylobacter jejuni	*Toxoplasma gondii*	Rotavirus	*Taenia saginata*
Escherichia coli		Enteroviruses	*Taenia solium*
		Reoviruses	*Necator americanus*
		Astroviruses	*Hymenolepis nana*
		Hepatitis E virus	
		Picobirnavirus	

[a]Pepper et al., 2006

1.4.1 Bacteria

Bacteria are considered to be prokaryotic, or organisms with no nucleus. They vary in size from 0.3 to 2 µm and takes shapes such as rods (*bacilli*), spheres (*cocci*), and spirals (*spirilla*) (Metcalf and Eddy, 2003 and Haas et al., 1999). There are two types of bacteria: gram-positive (non-pathogenic) and gram-negative (pathogenic). Bacteria are

usually present inside the body in the intestinal tract and are removed through the excrement of feces. They have the ability to infect humans and animals, but this is specific to certain strains of bacteria. Infection and illness from pathogenic bacteria occurs by either killing healthy cells directly or releasing toxins from the lipid-polysaccharides in their cell walls (Haas et al., 1999). The most common and widely studied bacterium is wastewater treatment is *Salmonella* which is responsible for typhoid fever (*Salmonella typhi*) and salmonellosis or food poisoning (*Salmonella paratyphy*) (Gerba et al., 2005; Metcalf and Eddy, 2003; Haas et al., 1999; and Straub et al., 1993). Other bacteria isolated from raw wastewater include *Shigella, Vibrio cholerae, Yersinia, Campylobacter jejuni,* and *Escherichia coli* (pathogenic strain). *Shigella* is known to infect only humans and cause bacillary dysentery. *Vibrio cholera* causes cholera which has symptoms of heavy diarrhea and dehydration (Metcalf and Eddy, 2003). Like *Shigella,* it is only known to infect humans generally through poorly sanitized water and is associated with the consumption of seafood. Feachem et al. (1983) showed that *Vibrio cholera* can persist in wastewater at low temperatures for long periods of time. This is probably due to the fact that the marine environment is its natural habitat (Haas et al., 1999). *Yersinia, Campylobacter jejuni,* and *Escherichia coli* are all known to cause gastroenteritis (Gerba et al., 2005). *Yersinia* has been reported to infect animals and humans and occasionally cause yersiniosis, the clinical name for its gastroenteritis, in the United States (Straub et al., 1993). It has been also reported to be found in raw, digested, and dewatered sludges (Metro, 1983). *Campylobacter jejuni* has been considered to be more widespread than *Salmonella* and *Shigella* (Archer and Kvenberg, 1985) and has been documented to infect animals and humans. *E. coli* are classified as opportunistic

pathogens which “are organisms that ordinarily do not cause disease in their natural habitat in normal health persons” (Haas et al., 1999). Opportunistic pathogens pose the most danger to persons whose health has already been affected by previous conditions such as burns, open sores, diabetes, AIDS, etc. (Haas et al., 1999). Pathogenic *E. coli* has been related to food and waterborne outbreaks (Feachem et al., 1983) and the O157:H strain is known to produce illness in humans (Haas et al., 1999). Other opportunistic pathogens that have been associated with drinking water include *Listeria* and *Legionella*. They have been reported to cause meningitis and pneumonia or Legionnaire’s disease, respectively (Haas et al., 1999), but mostly in developing countries.

1.4.2 Protozoa

Protozoa are classified as eukaryotes, organisms with a nucleus, and are more complex than prokaryotes (Haas et al., 1999). Some are non-pathogenic and naturally found in the environment, whereas others cause illnesses with symptoms such as diarrhea, stomach cramps, vomiting, nausea, and indigestion (Metacalf and Eddy, 2003). Illness-causing protozoans that have been related to water and/or food contamination are categorized into five groups: (1) ameobas (*Rhizopoda*), (2) flagellates (*Mastlgohora*), (3) ciliates (*Cilratea*), (4) *Sporozoa*, and (5) *Microsporidia*. Of the protozoans, the following have been more recently associated with sewage: *Entamoeba histolytica*, *Giardia lamblia*, *Balantidium coli*, and *Cryptosporidium* sp. (Straub et al., 1993). These protozoans are clinically important because they only reproduce in the host, typically in the intestinal tract, and spread infection from the excrement of cysts or oocysts (Haas et al., 1999). *Entamoeba histolytica*, *Giardia lamblia*, and *Balantidium coli* produce cysts while *Cryptosporidium* produce oocysts. Similar to *E. coli*, these protozoans have a

"significant impact on individuals with comprised immune systems" (Metcalf and Eddy, 2003). Oocysts are typically spherical in shape and range from 3 to 6 µm, whereas cysts can be spherical, semispherical, Ovid, tear drop, or kite-like, and range from 1 to 8 µm (Metcalf and Eddy, 2003). Viability of the oocysts and cysts depends on their ability to encyst (open up and grow) through the stomach within 18 hours (Haas et al., 1999). *Cryptosporidium* sp. and *Giardia lamblia* are of significant importance because they can cause disease in both animals and humans, have been found in nearly all wastewater streams, and sometimes survive after traditional wastewater and biosolids treatments (Metcalf and Eddy, 2003 and Haas et al., 1999). Their survival is attributed to their "protective structure called a *pellicle"* which covers the surrounding membrane (Haas et al., 1999).

1.4.3 Enteric Viruses

Enteric viruses are parasites that multiply or replicate in the intestinal tract and are released during the defecation of the infected host, either animal or human. "Viruses are usually 200 nm or smaller and cannot be seen with a light microscope" (Haas et al., 1999). Over 120 different types of viruses have been identified to cause numerous diseases such as respiratory infection and illness, eye infections, congenital heart disease, meningitis, myocarditis, gastroenteritis, hepatitis, and paralysis (Haas et al., 1999 and Straub et al., 1993). Enteroviruses, generally transmitted through aerosols and/or fecal matter, are the most commonly studied enteric viruses in sewage and sludge (Haas et al., 1999 and Straub et al., 1993). Enteroviruses include polio-, echo-, and coxsackie A- and B-, hepatitis A- and E-, reo-, rota-, and adenoviruses. Rotavirus and Norovirus (gastroenteritis), and hepatitis E (respiratory illness) have been related to several

waterborne outbreaks (Gerba et al., 1984 and 1985; Straub et al., 1993; and Haas et al., 1999). Reoviruses and adenoviruses have been isolated in wastewater and are known to cause respiratory illness, gastroenteritis, and eye infections (Metcalf and Eddy, 2003).

1.4.4 Helminth Ova

Helminths are worms and those that cause infection to humans are generally nematodes (roundworms) or platyhelminthes (flatworms) (Metcalf and Eddy, 2003). Helminth eggs, primarily the stage to cause human infection, range from 10 to 100 µm or more and can become concentrated in sludge (Metcalf and Eddy, 2003 and Feachem et al., 1983). Physical and chemical process such as sedimentation, filtration, and stabilization can reduce the concentration of helminth eggs in wastewater. However, *Ascaris* eggs were reported to survive 10 years in sediments of oxidation ponds (Nelson et al., 2001 as cited by Metcalf and Eddy, 2003); while *Taenia* eggs were not completely inactivated by sludge treatment processes (Feachem et al., 1983). *Ascaris lumbricoides* are roundworms that infect the small intestines (ascariasis) and are noted to be the most dominant parasite causing 1.5 billion infections worldwide (Crompton, 1999; Maier et al, 2000; and Roberts and Janovy, 1996 as cited by Metcalf and Eddy, 2003). In the United States alone, approximately 4 million cases were reported (Khuroo, 1996). *Taenia saginata and T. solium* are beef and pork tapeworms, respectively, which mainly cause illness from eating uncooked meat laden with tapeworm eggs (Straub et al., 1993). Symptoms of infection include abdominal pain, weight loss, and digestive disturbances (Straub et al., 1983). *T. saginata* tapeworms are the most popular in humans (Metcalf and Eddy, 2003). Similar to the roundworm is the whipworm, *Trichuris trichiura*, which infects the large intestines (trichuriasis) with symptoms of abdominal pain and diarrhea, and damages the internal

organs with increasing concentrations (Straub et al, 1993). *Necator americanus* and *Ancyclostoma duodenale* (hookworms) and *Strongyloides stercoralis* (threadworms) are other nematodes that cause human infection (Metcalf and Eddy, 2003). *N. americanus* and *A. duodenale* generally infect the small intestines (ancyclostomiasis) with occasional symptoms of anemia and debility infection (Straub et al., 1993). Feachem et al. (1983) indicated that hookworms tend to be less resistant than *Ascaris* eggs.

1.4.5 Indicator Organisms

Due to the difficulty of separating and identifying pathogens found in biosolids, indicator microorganisms can be used to detect the presence of pathogens. An ideal indicator should meet the following criteria: (1) consistently present when fecal contamination is present;(2) have an equal or greater presence than pathogens, (3) display the same resistance to the environment as pathogens, (4) cannot multiply outside the host organism, (5) possess faster, easier, and cheaper detection and quantification methods than pathogens, and (6) prone to be located in the intestinal tract of warm blooded animals (Cooper, 2001 and Maier et al., 2001 as cited by Metcalf and Eddy, 2003). Although no organisms have been found to exhibit all the characteristics of an ideal indicator, some of the organisms that have been used as *surrogates* for pathogens in biosolids include total and fecal coliform bacteria, *E. coli*, bacteriodes, fecal streptococci, enterococci, *Clostridium perfringens*, and coliphage. Coliform bacteria are one of the most common indicators of fecal contamination. This is due to fact that they are naturally located in the human intestinal tract and approximately 100 to 400 billion are released by each person a day (Metcalf and Eddy, 2003). Fecal coliforms are a subset of total coliforms that can colonize at higher temperatures (45.5 ± 0.2°C) than total

coliforms (35 ± 0.5°C) (Metcalf and Eddy, 2003). *E. coli* is another coliform bacterium that has been reported to be more indicative of fecal contamination (Metcalf and Eddy, 2003). Alternative bacterial indicators have become more popular due the limitations of coliforms. Some concerns with coliforms have been their inability to detect the presence of enteric viruses and protozoa, short survival time, and possibility of regrowth outside the host organism (Metcalf and Eddy, 2003 and Sidhu and Toze, 2009). Bacteriodes, fecal streptococci, and enterococci can differentiate between humans and animal feces which make them useful in fecal pollution source tracking (Siduh and Toze, 2009). *Clostridium perfringens* spores are used to indicate the presence of protozoa like *Cryptosporidium* and *Giardia*, but their effectiveness is disputable (Sidhu and Toze, 2009). Coliphage, mainly somatic and male-specific (F+), are members of bacteriophages which are used to indicate enteric viruses (Metcalf and Eddy, 2003 and Sidhu and Toze, 2009). Since no one organism is perfect in determining the presence of pathogens in biosolids, it is suggested that a combination of indictor organisms proves more useful and effective (Straub et al., 1993).

1.5 Hazardous Characteristics of Pathogens

There are a wide variety of pathogens potentially present in biosolids, and the hazards presented by these pathogens vary based on their initial concentration, dose-response behavior, and survival. To assess the hazards presented by pathogens, quantitative microbial risk assessment (QMRA) is used. QMRA along with the general framework of risk assessment and its uncertainties are discussed in further detail in the next chapter. This section specifically focuses on three characteristics that make pathogens in biosolids hazardous.

1.5.1 Occurrence

The occurrence of pathogens in wastewater treatment plants depends on season, climate, and sanitation (Straub et. al., 1993). With the progression in sanitation methods and continuous scrutiny of water quality standards, occurrence of pathogens in untreated sludge is expected to increase and become more concentrated. This is due to the fact that "many microorganisms are known to survive better in wastewater when they are associated with the solid particles rather than in suspended state" (Sidhu and Toze, 2009). Ward et al. (1984) showed that various enteric viruses, *Salmonella* sp., *Giardia* sp., and Helminths typically ranged from 10^2 to 10^3 organisms/g of dry weight in treated secondary sludges. The study also specified that indicators such as total and fecal coliforms ranged from 10^6 to 10^8 organisms/g of dry weight. Anaerobic digestion produced *Salmonella* sp. densities of 0.8 to 33 MPN/g (Farrah and Bitton, 1984) and appeared to be ineffective on Ascaris in another study (Pedersen, 1981). However, composting, a method denoted to further reduce pathogens, adequately reduced enteric viruses and helminth concentrations as to not pose a threat to human health (Yanko, 1988). A 3 to 4 log reduction in enteric viruses, indicator bacteria and possibly helminth can be expected from composting in the thermophilic temperature range (Straub et al., 1993). Composting does have the potential to support regrowth of pathogenic bacteria such as *Salmonella* sp., *Yersinia* enterocolitica and toxigenic E. coli (Yanko, 1988). Before the 1990s, detection and monitoring of pathogens in sludge was poorly conducted. There was lack of research on pathogen fate and transport as well as techniques to successfully recovery pathogens from the soil and water environment (Straub et al, 1993). Establishment of the EPA 1993 rule to disposal of sludge sparked much interest and

research continues to flourish in this field. New technologies for the detection of pathogens and effective treatments to reduce pathogen concentration in biosolids have been conducted. For example, Chauret et al. (1999) studied the effectiveness of aerobic wastewater treatment and anaerobic digestion of sludge to reduce selected microorganisms and remove pathogenic protozoa in a wastewater treatment plant in Ottawa, Canada. Samples were collected from raw and treated wastewater, primary effluent, mixed sludge, decanted liquor, and sludge cake. Bacterial and somatic coliphage enumerations and fluorescent antibody staining were used to detect the presence of microorganisms and pathogens. Results showed that all raw samples were positive for tested microorganisms, as well as *Cryptosporidium* oocysts and *Giardia* cysts. Aerobic treatment reduced *Cryptosporidium* oocysts and *Giardia* cysts by 2.96 and 1.40 logs, respectively, in treated wastewater. *Clostridium perfringens* spores and total count, somatic coliphage, and heterotrophic bacteria were reduced by approximately 0.89, 0.96, 1.58 and 2.02 logs, respectively. Remaining microorganisms had at least a 3.53 log reduction. Anaerobic digestion of the sludge, however, only reduced fecal coliforms and bacteria by mostly 2 logs. Reduction of *Cryptosporidium* oocysts, *Giardia* cysts, *Clostridium*, and *Enterococcus* sp. were not statistically significantly reduced. Likewise, Graczyk et al. (2006) showed that *Cryptosporidium* oocysts, *Giardia* cysts, and human-virulent microsporidia spores were detectable in activated sewage sludge. Multiplex fluorescence in situ hybridization (FISH) assay and direct immunofluorescence assay were used to detect the pathogens. *Cryptosporidium* oocysts, *Giardia* cysts, and human-virulent microsporidia spores had an average concentration of 338 oocysts/liter (L), 843 cysts/L, and 224 spores/L, respectively. Dewatering and biological stabilization

processes further reduced these concentrations to 88% less than in activated sludge. Guzman et al. (2007) looked at the persistence of helminth ova, *Cryptosporidium* sp., *Salmonella* spp., enteroviruses, and bacterial and viral indicators in raw and treated sludges. Sludge treatment processes included mesophilic and thermophilic digestion and composting. Low concentrations of helminth ova were detected in all samples, and viable *Cryptosporidium* oocysts were still present in treated sludges. Fecal coliforms, sulphite-reducing clostridia (SSRC), and somatic coliphages were the only indicators with values above their detection limits in most of the samples. Results from these few current studies were similar to the densities of pathogen and indicators in secondary treated sludges. A 2009 literature review was conducted by Sidhu and Toze that complied data on the occurrence of pathogens expected in biosolids. It was noted that concentration of indicator organisms were anticipated to be 2 to 3 orders of magnitudes higher than that of pathogenic bacteria, while protozoan organisms were low (Sidhu and Toze, 2009). Bacteriophage concentrations were similar to that of enteric viruses (Sidhu and Toze, 2009). Although, the compilation was a great effort, Sidhu and Toze data mostly targets occurrence of biosolids outside the United States. Only one reference (Dahab and Surampalli, 2002) provided information on the treatment of biosolids in the United States. Dahab and Surampalli (2002) indicated that the concentration of fecal coliforms, fecal streptococcus, and Salmonella averaged approximately 3.6×10^{7}, 2.1×10^{7}, and 6.2×10^{2} organisms/g in United Stated dried sludge. A more recent study was performed to reflect the advancement of sludge treatment in the United States. Pepper et al. (2010) conducted “the first major study of its kind since the promulgation of the USEPA Part 503 Rule in 1993”. This research initially studied the occurrence of indicator

organisms and pathogens found in Class B Biosolids. These biosolids were subjected to mesophilic, anaerobic digestion. Twenty-one (21) samples were collected from 18 different wastewater facilities in the United States. Results showed that bacterial and viral pathogens in Class B biosolids were generally low; however adenoviruses were more common than enteric viruses. High concentrations of both bacterial and viral indicator organism numbers were seen. Toxigenic E. coli (E. coli 0157:H7), *Campylobacter*, and Ascaris ova were all below detection. Secondly, the study reviewed the historic distributions of data from 1988 and 2006 at one location in Tucson, AZ. It was concluded that pathogens and indictor organisms reduced by 94 to 99% over the 18 year period. "Presumably this is due to better and more consistent treatment of the wastewater, illustrating that the Part 503 Rule has been effective in reducing public exposure to pathogens" in land applied biosolids (Pepper et al, 2010). Although research support the fact that occurrence of pathogens has tended to decrease, pathogens are still being detected in some treated sludge samples. Effective detection, identification, and removal are the only way to prevent the spread of pathogens in the environment and infection to the public from land applied biosolids.

1.5.2 Dose-Response

Paracelsus, a 16th century chemist and physician, first stated the often quoted "the dose makes the poison." This expresses the concept that the amount of exposure determines if there will be an adverse outcome. Dose-response can be described as the relationship between the dose given and the elicited health effect. Teunis et al. (1996) best explains the dose-response for a given effect as "the quantitative relation between the *intensity* of exposure (the dose) and the *frequency* of the occurrence of this effect within the exposed

population of hosts (the response)". Dose is the amount of chemical (or pathogen in this research) ultimately delivered to an organ tissue. Microbial doses are based on methods "routinely used to count specific microbe in the laboratory" (Haas et al., 1999). Microbial dose can be expressed as colony-forming particles on agar media for bacteria, plaque-forming particles for viruses, and direct microscopic count of spores and (oo)cysts for protozoa (Teunis et al, 1996; Haas et al., 1999). Dose-response curves are modeled with the logarithm of dose plotted against the level of response, which can be used in the threshold concept of no-effect levels (Haas, 1983). Major routes of exposure to organ tissue include inhalation, ingestion, dermal, and injection. Human and animal exposure to infectious pathogens is possible during land application of biosolids. Aerosolized biosolids sprayed onto agricultural soil can effectively transport enteric microorganisms (Pahren and Jakubowski, 1980) and be inhaled from the wind and physically deposited onto the skin (Straub et al., 1993). Also, pathogens can be directly ingested by the consumption of soils amended with biosolids. Indirectly, there is the potential to ingest contaminated ground water or near surface waters from leachate and runoff that may transport soil particles amended with biosolids (Straub et al., 1993). Ingestion of pathogens, specifically a variety of bacteria, viruses, and parasites, can lead to food- and waterborne illness such as gastroenteritis. Gastroenteritis is a major cause of disease and death worldwide and is mostly seen in young children in developing countries (Benenson, 1990). In the United States, it is the most common waterborne illness and the second most common illness to cause acute disability (Craun, 1991 and Monto et al., 1983). An indication of infection is sometimes seen with symptoms such as diarrhea, fever, and vomiting; however, it is not necessary. Usually with increasing dose, the

biological effect or response is greater. The overall objective of a dose-response assessment is to determine the likelihood of a dose under real life conditions leading to infection or death. This research is particularly interested in the possibility of infection given exposure to pathogen by ingestion of biosolids. Modeling of dose-response relationships was first studied by Haas (1983) and subsequently thereafter (Rose et al., 1991; Haas et al., 1993; and Regli et al., 1993). Their research established the foundation of dose-response modeling and is still used today (Teunis et al., 1996; Haas et al., 1999; Soller et al., 2004; Armstrong et al., 2007; Smith et al., 2008; and Navarro et al., 2009). Dose-response models are determined from epidemiological studies on human subjects and toxicological studies of nonhuman subjects. Under real life conditions, the dose to cause infection is relatively low and must be extrapolated from the high doses often utilized in these studies. Exponential or Beta-Poisson models have been studied on various organisms and have shown to best-fit dose-response data (Haas, 1993). These models are based on the assumption that organisms are randomly distributed within the consumed medium (biosolids) and follow a Poisson distribution. This is due to the fact that "each organism has an independent and identical survival probability" to infect its host (Haas et al., 1999). The exponential dose-response model can be expressed as the following:

$$R = 1 - e^{(-\text{dose} \times r)}, \quad (1)$$

where dose (organisms) is the amount of organisms swallowed and r (organisms^{-1}) is the exponential dose-response parameter. The Beta-Poisson model in microbial dose-response relationships was derived by Furumoto and Mickey (1967, as cited by Haas et al., 1999) and is given by $R = 1 - (1 + \text{dose}/\beta)^{-\alpha}$, where α and β are the parameters to

adjust the model to fit dose-response data. Variables that affect the dose are exposure concentration, length of exposure, and body weight of the exposed subject. Much discussion has been on the time to onset effect. Researchers have often reported the time needed to produce a given affect as either requiring a single dose or several doses to initiate infection (Blaser et al., 1982; Haas, 1983; and Rubin, 1987). It was concluded that exponential and Beta-Poisson models support the fact that "each microorganism is capable of infection" and are an improvement over the traditional lognormal model (Haas et al., 1999). Another issue to consider is the use of animal models to extrapolate low doses and prolonged exposure times in humans. A large sample size would be needed to determine the probability of infection at such low doses, and it would be extremely expensive "to provide adequate treatment to all the subjects involved" (Teunis et al., 1996). Extrapolation to low doses tends to overestimate risk in real-life situations making it a conservative, yet uncertain estimate. As stated by Haas et al. (1999), more sufficient data from animals and humans is needed to accurately "address both hazard and dose-response, including virulence, strain variation and immunity, and multiple exposures".

1.5.3 Survival

Survival of pathogens is one of the most important parameters in determining the hazard of pathogens. In terms of biosolids, survival consists of the persistence of pathogens after physical, chemical, and biologically processes failed to inactivate pathogens. Survival can be quantified as the rate of decay (k) in $hours^{-1}$ or $days^{-1}$. Temperature is the key factor in determining survival of viruses, bacteria, protoza, and helminth (Straub, 1993). Ahmed and Sorensen (1995) studied the effects of stored digested and dewatered

biosolids to determine the kinetics of pathogen destruction. Biosolids were collected from a wastewater treatment plant in central Utah. Treatment processes consisted of primary settling, trickling filter, and aerobic and anaerobic digestion. Biosolids were then stored for approximately 62 days to further reduce pathogens at various temperatures: 5, 22, 38, and 49.5°C (41, 72, 100, and 121°F). Results showed that destruction of pathogens occurred at all temperatures examined; however, decay rates increased with increasing temperature. There was no significant difference between the destruction of pathogens under aerobic or anaerobic conditions at all temperatures studied. At 50°C, the decay rate of *S. typhimurium*, *Y. enterocolitica*, bacteriophage f2, poliovirus, and *Ascaris* eggs was estimated to be 1.13, 1.10, 1.54, 0.81, and 0.21 log reduction/d^{-1}, respectively. With viruses, soil moisture plays a role in its survival. Research has shown when high temperatures and evaporation decrease water content, rapid deactivation of viruses occurs (Gerba et al., 1981; Hurst et al., 1980a,b; Bitton et al., 1984; and Straub et al., 1992, 1993). In addition to temperature, survival of bacteria is also influenced by bioavailability of nutrients, pH, and moisture. These factors have the greatest impact on bacterial survival (Gerba et al., 1975). Pepper et al. (1991) showed that bacterial indicator organisms were not detected on cotton farms until after land application of biosolids. This could be attributed to Mallman and Litsky (1951) suggestion that sludge organic content may improve bacterial survival. Helminth ova, specifically *Ascaris* eggs, have been determined to be the most persistent pathogen after land application (Cram, 1943; Jackson et al., 1977; and Meyer et al., 1978). Composting of biowaste was studied to determine its effect on the survival of *Listeria* monocytogenes, *Salmonella* sp., and *Escherichia coli* (Lemunier et al., 2005). *E. coli* and

L. monocytogenes survived in compost up to 4 weeks old; however, strains of *Salmonella* survived in all composts at longer periods of time (up to 3 months). Long-term storage of dewatered, digested and composted biosolids can be effective in reducing the risk of infection when average ambient air temperatures are 20°C (68°F) or higher. Increase in temperature of biosolids shortens the timeframe to prevent survival of pathogens and further improve the quality of the biosolids. However, inappropriate storage during the winter months and deep burial of biosolids inhibit proper evaporation (Straub, 1993). As a result, microbial risk assessments must be conducted with the most current data available and as little error as possible to properly protect human health and the environment.

CHAPTER 2: RISK ASSESSMENT AND ITS UNCERTAINTEIS

Risk assessment is defined as the "qualitative or quantitative characterization and estimation of potential adverse health effects associated with exposure of individuals or populations to hazards (materials or situations, physical, chemical and or microbial agents)" (Haas et al., 1999). In simple terms, risk assessment tries to (1) identify the item that may potentially cause harm; (2) measure the likelihood of a negative outcome occurring; and (3) determine the magnitude of the effect the outcome could deliver. Risk assessment is an entire concept within itself; however it falls under the umbrella of Risk analysis. Risk analysis is also composed of risk management and risk communication. Risk management is the control of risks and generally involves selecting and implementing control measures. It not only takes into consideration quantitative risks, but also policy making, ethics, engineering, and cost-benefits (Haas et al., 1999). Risk communication is the exchange of scientific information in a balanced discussion among experts and the public. These three concepts of risk analysis were initially assumed to be distinct entities with a firewall that separated assessment and management. This was known as the "Red Book" approach that formally established the field of risk assessment in an environmental setting (NRC, 1983). More recent studies by the NRC showed that this wall was impractical and that risk analysis should involve all "interested and affected parties" throughout the process (NRC, 1996). Although the firewall is out, the 1983 framework for risk assessment is still being used. Figure 2.1 is a schematic of the risk assessment paradigm which involves a sequence of four steps.

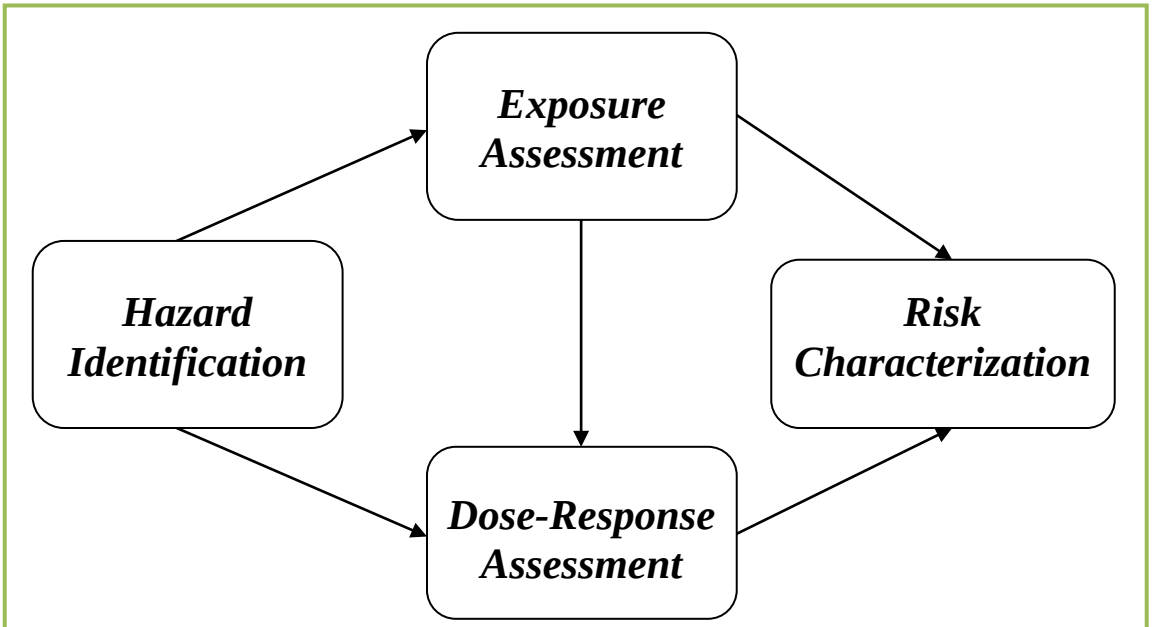

Figure 2.1 **Schematic of the Risk Assessment Framework.**

The first step is hazard identification which focuses on pinpointing the hazard and its potential to cause harm. It includes developing options for eliminating or substituting less toxic chemicals, hazard control measures, and responsible reuse or disposal of chemicals. The second step is the exposure assessment which determines the concentration and intake rate of a contaminant to produce an adverse health effect and toxicity to an organism. It also determines the pathways and duration of the exposure. The next step is the dose-response assessment which quantifies the occurrence of health effects based on the degree of exposure. It is usually described as a mathematical expression which plots the response versus dose. The last step is risk characterization which determines the severity of a hazard and its variability and uncertainty from the information in the previous three steps. Risk assessment can be applied in different areas

such as finance, insurance, health and safety, and ecology. This research is interested in health based risks which was best described by Gerba (c.2007).

> For these risks, the focus is on general human health, mainly outside the workplace. Health-based risks typically involve high-probability, low-consequence, chronic exposures whose long latency periods and delayed effects make cause-and-effect relationships difficult to establish. This category also includes microbial risks, which usually have acute short-term effects. However, the consequences of microbial infection can persist throughout an individual's lifetime.

To assess the hazards presented by pathogens, quantitative microbial risk assessment (QMRA) is used. QMRA seeks to estimate the potential effects on human health associated with exposure to microbial agents. QMRA identifies specific microorganisms and routes of exposure, develops dose-response models, examine host-microorganism interactions all while effectively incorporating epidemiological studies to assess the health of the exposed (Haas et. al., 1999). Risk assessment of microorganisms was initially studied in waterborne pathogens, especially due to the outbreak of infectious diseases and illnesses common in contaminated water. These assessments yielded qualitative information on risks but did not explain the "microbial agents, their concentrations, distributions, and sources, and the potential for other serious or chronic health effects" (Haas et al., 1999). Haas (1983), Rose et al. (1991), and Regli et al. (1991) were some of the first scientists to develop dose-response models and quantitative risks in in drinking water. Microbial agents have become extremely important since the use of biosolids as fertilizer in farming and landscaping has increased over the past 20

years (Eisenberg et al., 2006). As a means to protect the public health and the environment from the negative effects of pollutants in biosolids, the U.S. EPA established the final rule of the Part 503 Standards for the Use and Disposal of Sewage Sludge in 1993 (EPA, 1993). Technological based standards and management and record keeping were the avenues used to limit pathogens in biosolids before the 1993 final rule. This rule allowed for limits on chemical compositions in biosolids to be based on quantitative risk assessment. Microbial risk assessment techniques were thought to be novel and not properly developed. However, "microbial risk assessment has progressed substantially in the past 15 years" (Haas et al., 1999; Eisenberg et al., 2002; and Soller et al., 2006). The NRC suggested expanding risk assessment methods to institute more restrictive limits for chemical and pathogen in biosolids (NRC, 2000). However, due to insufficient and antiquated data, parameters to quantify risk of infection from pathogens might have extensive uncertainty (Haas, 1999). Limitation in the hazard identification step is due the unavailability of extensive epidemiological data, case studies, and disease investigations. More "accurate methods for recovery, detection, quantification, sensitivity, specificity, virulence, and viability, as well as studies and models addressing transport and fate through the environment" are required to develop exhaustive databases for dose-response and exposure assessments (Haas et al., 1999). Another source of uncertainty is the substitution of dose-response and occurrence of related microorganisms for new pathogens where data is non-existing. Most of the literature available on concentration, survival time, and dose response of pathogens in biosolids were published before the EPA final rule for Standards for the Use and Disposal of Sewage Sludge (Ahmed, 1997). Since 1993, occurrence of pathogens in Class B biosolids has generally

decreased; however, occurrence, a key element in determining exposure, has been attributed to render the most uncertainty in risk characterization (Haas et al., 1999).

CHAPTER 3: METHODOLOGY

3.1 Derivation of Relative Risk Metric Equations

Recall Eq. (1) where risk R due to biosolids if ingested at time $t = 0$ days is $R = 1 - e^{(-\text{dose} \times r)}$, where dose (organisms) is the amount of organisms swallowed and r (organisms^{-1}) is the exponential dose-response. A Taylor Series can be used to approximate the dose-response function when risk is low as $R = r \times$ dose, and dose can be expressed by $C_0 \times$ Uptake, where C_0 (organisms/gram (g)) is the pathogenic concentration in biosolids and Uptake (g/day (d)) is the amount of biosolids ingested per day. Thus, low risk can be approximated as $R = r \times C_0 \times$ Uptake. Supposing that a given exposure is repeated on a daily basis and pathogenic concentrations decay exponentially, risk at subsequent time periods may be expressed as

$$R = r \times C_0 \times \text{Uptake} \times e^{-kt}, \tag{2}$$

where k (day^{-1}) is the decay parameter. Maintaining the assumption of low risks allows risks on subsequent days to be summed based on the following justification. The union of two risks is the sum of the two risks minus the intersection. For independent risks, the intersection will be the product of the two risks. If both risks are small, then this intersection will be smaller still (e.g. if both risks are on the order of 10^{-2}, then the intersection will be on the order of 10^{-4}, or two orders of magnitude smaller). The summation of risks over time may be carried out continuously by integration. Integrating Eq. (2) from $t = 0$ to $t = \infty$ yields the following:

$$\int_{t=0}^{t=\infty} (r \times C_0 \times Uptake \times e^{-kt}) dt$$

$$= r \times C_0 \times \text{Uptake} \int_{t=0}^{t=\infty} e^{-kt} dt$$

$$= r \times C_0 \times \text{Uptake} \times \left[\frac{e^{-kt}}{-k} \right]_{t=0}^{t=\infty}$$

$$= r \times C_0 \times \text{Uptake} \times \left[0 - \frac{1}{-k} \right]$$

$$R = r \times C_0 \times \text{Uptake} \times k^{-1}. \quad (3)$$

Uptake can be omitted from Eq. (3) since this research is interested in a relative risk metric and uptake will be constant across different pathogens. This simplifies Eq. (3) to

$$RRMO = r \times C_0 \times k^{-1}, \quad (4)$$

where *RRMO* is the dimensionless relative risk metric conditioned on occurrence. Given that data on occurrence of pathogens in biosolids after the 1993 EPA 40 CFR Part 503 is limited, an estimated risk metric without occurrence (RRM) can be determined by

$$RRM = \frac{r}{k}. \quad (5)$$

3.2 Criteria for Inclusion

An extensive literature search was conducted to obtain numerical data on the three relative risk parameters mentioned in Eq. (4) for pathogens commonly found in Class B biosolids. Data for each parameter were selected only if appropriate descriptive statistics were available. For concentration, this research specifically focused on mesophilic anaerobic digested (MAD) biosolids. MAD treatment stabilizes biosolids in a temperature range between 30 and 38°C (85 and 100°F) (Metcalf and Eddy, 2003). This method of treatment was selected because it is the "most widely used" system to reduce pathogenic concentrations (Ponugati, 1997). Ward (1984) reported that bacteria concentrations are reduced by 0.5 to 4 $\log_{10}$, viruses by 0.5 to 2 $\log_{10}$, and parasites by less than 0.5 $\log_{10}$ units when mesophilic anaerobic or aerobic digestion processes were utilized. Only concentrations determined by

cell culture methods were selected. Exponential or Beta-Poisson dose-response values based on human subjects were the most preferred. However, animal dose-response values were utilized when human values were not available or did not include the required descriptive statistics. Microbial decay values were collected from the best data available. Values were based on decay in various water sources in suspended form and temperatures ranging from 0 to 55° C (32 to 131°F). Sources of water included but were not limited to rivers, groundwater, freshwater, wells, seawater, and distilled water. Decay in effluent, composted manure, and soil were also used when applicable.

3.3 Initial Calculations

Initial calculations for all risk parameters included determining the 90^{th} percentile upper bound (UB) or lower bound (LB) for each pathogen. Other percentiles were calculated if confidence intervals were already indicated. Figure 3.1 shows the process that was followed to identify values for each parameter. Concentration and dose-response uncertainty was based on the upper bound (*UB*), whereas uncertainty for decay was based on the lower bound (*LB*). If the *UB* or *LB* was not reported, a factor of 10 above or below the mean was applied to determine the *UB* and *LB*, respectively. In the absence of a reported standard deviation (σ) for concentration, decay, and dose-response parameters, the following equation was used:

$$\sigma_{\ln} = \frac{\ln UB - \ln \text{nominal}}{Z_{\alpha}}, \tag{6}$$

where nominal is the mean or central tendency, and Z_{α} is the z-statistic (1 - α) percentile. If α was not given, a value of 0.1 was assumed. These values will determine a conservative upper bound of calculated relative risks. All concentration values were converted to organisms/g, if necessary. As stated above, data for the dose-response parameter was preferred to be modeled as Exponential or Beta-Poisson as studies have shown that these

models tend to best-fit dose-response data (Haas, 1993). Since this research focuses on low risk, the Beta-Poisson dose-response function can be approximated by a Taylor series as

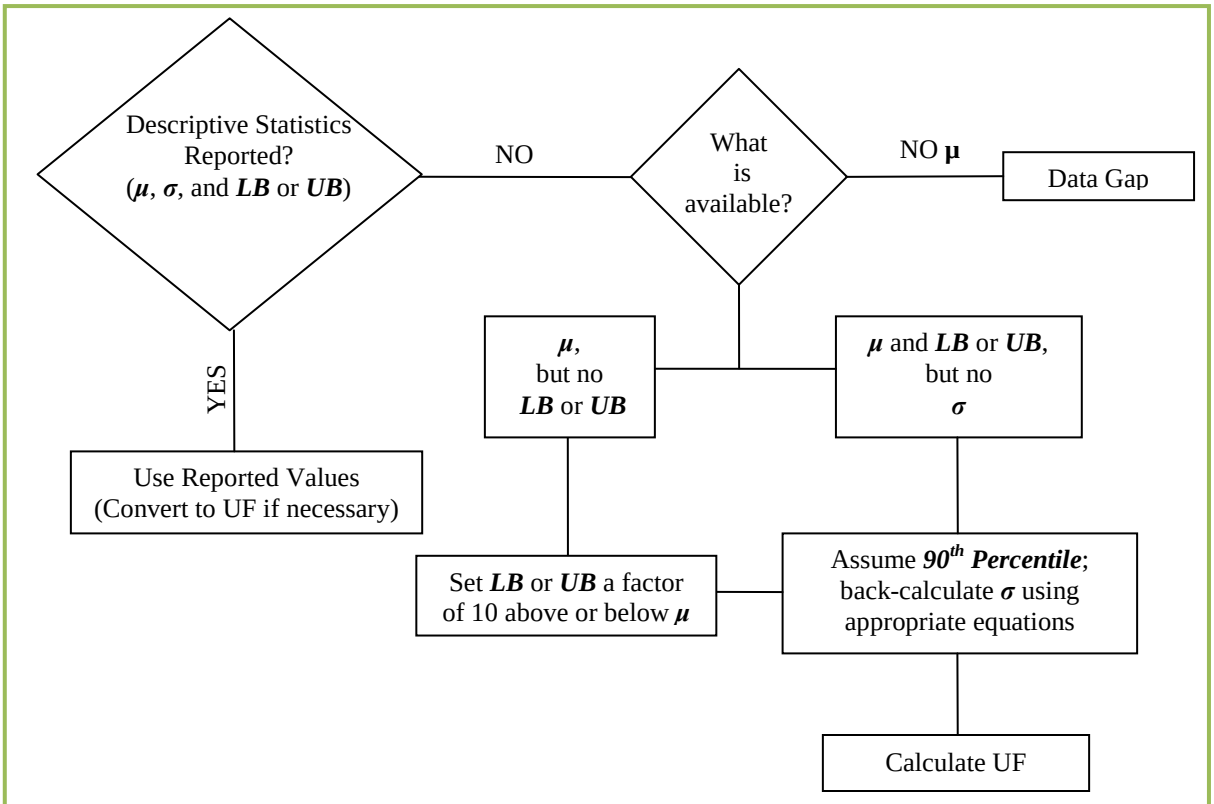

Figure 3.1 **Flowchart of Parameterization.**

(α/β) × dose, where α and β are parameters for the Beta-Poisson model. This is the same as the exponential low dose approximation given previously but with $r = \alpha/\beta$. Other dose-response relationships were also used to estimate r when adequate. If the mean dose (N_{50}) or the mean lethal dose (LD_{50}) was given, r was approximated from $0.5 = 1 - e^{(-LD_{50} \times r)}$. When the exponential k was given, r was calculated as $r = 1/k$. Once all descriptive statistics were

accounted for, the uncertainty factor (UF) was determined for each parameter when applicable as UF = *UB*/nominal or UF = *LB* × nominal.

3.4 Point Estimated Risks

An initial view of the relative risk of infection was determined for pathogens encompassing values for each risk parameter. Mean concentration, dose-response, and decay values for qualifying pathogens were substituted into Eq. (4). Based on the output generated for the *RRMO*, pathogens were classified as presenting high to low relative risk of infection. Subsequently, the *RRM* was calculated using the mean values of dose-response and decay parameters. The 90 percent confidence intervals were determined for both risk metrics from the variance calculated in the next section. Microsoft Excel 2010 was used to find the distribution that best fit the *RRMO* and the *RRM*.

3.5 Uncertainty Analysis

Uncertainty in the relative risk metrics was assessed analytically. Taking the logs of both sides of Eq. (4) yielded ln *RRMO* = ln *r* + ln C_0 - ln *k*. Thus, the natural log of the *RRMO* is the sum of random variables and the variance of this sum is given by

$$\sigma^2_{\ln RRMO} = \sigma^2_{\ln r} + \sigma^2_{\ln C_0} + \sigma^2_{\ln k} + 2\,\mathrm{cov}(r, C_0) + 2\,\mathrm{cov}(r, k) + 2\,\mathrm{cov}(C_0, k), \qquad (7)$$

where cov is the covariance. Since the random variables are independent, cov = 0, and thus these covariance terms are eliminated. Therefore, Eq. (7) simplifies to

$$\sigma^2_{\ln RRMO} = \sigma^2_{\ln r} + \sigma^2_{\ln C_0} + \sigma^2_{\ln k}\,. \qquad (8)$$

If all descriptive statistics were available for a particular parameter, values were log-transformed and the standard deviation of the logs ($\sigma_{\ln}$) was back-calculated with *Solver* in Microsoft Excel 2010 using the following equation:

$$\mathrm{Variance} = \left(e^{\sigma_{\ln}{}^2} - 1\right) e^{2\mu_{\ln} + \sigma_{\ln}{}^2}. \qquad (9)$$

The solved σ_{ln} was then squared to obtain the variance of the log-parameter. If σ was not reported, σ_{ln} for the parameter was back-calculated from Eq. 6 and then squared to obtain the variance. Contribution of parameter with most uncertainty was determined as a percentage the parameter variance versus the risk metric variance:

$$\text{Max Uncertainty } RRMO = \max\left[\frac{\sigma^2_{\ln r}}{\sigma^2_{\ln RRMO}}, \frac{\sigma^2_{\ln C_0}}{\sigma^2_{\ln RRMO}}, \frac{\sigma^2_{\ln k}}{\sigma^2_{\ln RRMO}}\right]. \quad (10)$$

The same was performed to determine uncertainty in the *RRM*. Taking the logs of both sides of Eq. (5) yielded ln *RRM* = ln *r* - ln *k*. Thus, the natural log of the *RRM* is the sum of random variables and the variance of this sum is given by

$$\sigma^2_{\ln RRMO} = \sigma^2_{\ln r} + \sigma^2_{\ln k} + 2\,\mathrm{cov}(r,k)\,. \quad (11)$$

Since the random variables are independent, the covariance term is eliminated and Eq. 11 simplifies to Eq. 12:

$$\sigma^2_{\ln RRM} = \sigma^2_{\ln r} + \sigma^2_{\ln k}\,. \quad (12)$$

Identification of the parameter that contributed the most uncertainty in the *RRM* was also determined as a percentage of the variance of the risk parameters versus the variance of the *RRM*:

$$\text{Max Uncertainty } RRM = \max\left[\frac{\sigma^2_{\ln r}}{\sigma^2_{\ln RRM}}, \frac{\sigma^2_{\ln k}}{\sigma^2_{\ln RRM}}\right]. \quad (13)$$

CHAPTER 4: RESULTS AND DISCUSSION

4.1. Collection of Numerical Data

4.1.1. Occurrence

Quantitative data was collected on the following parameters to determine the relative risk of infection from pathogens in Class B Biosolids: (1) occurrence, (2) dose-response, and (3) decay. Data on the occurrence of pathogens and indicators that met the required criteria were only available for 14 organisms. Appendix B summarizes the reported literature on the occurrence of these pathogens. Information was first obtained from the Pepper et al. (2010) study as it consisted of the most recent concentrations of several organisms in United States MAD biosolids, and immediate access to the original data was available. From 2005 to 2008, 21 samples were collected from 18 treatment plants which catered to approximately 500,000 residents each around the county (Pepper et al., 2010). Pathogen concentrations were obtained from either cake (20% solids) or slurry (8% solids) samples using various assay methods, and were fitted to a lognormal distribution with maximum likelihood estimation (MLE) (Pepper et al., 2010). Results showed that *Campylobacter*, *E. coli* O157:H, and Ascaris were non-detectable, while *Shigella* and enteric viruses were at low concentrations. However, incidence of Adenovirus and indicator organisms such as fecal streptococci, *Clostridium perfringens*, fecal and total coliforms, and somatic Coliphage was high. Statistical data on the occurrence of *Cryptosporidium*, *Giardia*, and *E. coli* were provided by Guzman et al. (2007), Chauret et al. (1999), and Wong et al. (2010), respectively. In 2005, Guzman et al. (2007) analyzed six samples of incoming and outgoing sludges from two treatment plants in Barcelona, Spain. Plant 1 was of interest as it consisted of a mixture of raw and secondary sludge

subjected to MAD for 20 to 25 days and produced a dewatered sludge of 25% dry matter (d.m.). Mean concentration of viable *Cryptosporidium* oocysts was reported as 20 per 10 g of d.m and ranged from 0.74 to 0.67 (Guzman et al., 2007). It was believed that the max value given was a typo and rather should be 6.7×10^1 as research has shown that MAD yields a 1 to 2 log reduction of bacteria and viruses (Pedersen, 1981). In 1995, Chauret et al. (1999) collected 10 semisolid cake samples of sludge also treated by MAD for 20 days from a wastewater treatment plant in Ottawa-Carleton, Canada. Results for *Giardia* cysts showed a 1.40 log reduction with a mean average of 1.28×10^3 per 100 g of wet sludge (counts corrected to account for dewatering) (Chauret et al., 1995). Concentrations were as low as below detection to as high as 2.82×10^3 per 100 g. Wong et al. (2010) collected 12 MAD biosolid samples from four wastewater treatment plants in Michigan from 2008 to 2009. A log reduction of 1.5 was seen for *E. coli* with an extrapolated mean concentration of 3.16×10^3 organisms/g. The concentration ranged from 6.05 to 1.12×10^6 organisms/g. Each study mentioned above looked at the occurrence of similar organisms in MAD biosolids; however, only the most recent and useful values were selected for this research.

4.1.2. Dose-Response

Collection of data for the dose-response parameter was more successful than that of occurrence. The numerical compilation of the data can be found in Appendix C. Human exponential dose-responses were found for the following pathogens: *Cryptosporidium*, *Giardia*, Adenovirus type 4, Hepatitis A virus, Astrovirus, and Norovirus (Haas et al., 1999; Teunis et al., 1996; Couch et al., 1966; Ward et al., 1958; and Teunis et al., 2008). Viruses tend to fit exponential data well due to the low number of human subjects and

doses administered; however, protozoa like *Cryptosporidium* and *Giardia* fit regardless of the number of volunteers and doses (Haas et al., 1999). Human dose-response data for *Campylobacter*, *E. coli* O157:H, *Salmonella*, *Shigella*, *Vibrio cholerae*, *Ascaris*, enteric viruses, and Rotavirus were reported to best-fit beta-Poisson models (Teunis et al., 1996; Powell et al., 2000; Teunis et al., 1999; Soller et al., 2004; Haas et al., 1999; Navarro et al., 2009; Ward et al., 1986; Regli et al., 1991; and Haas et al., 1993). Enteric bacteria have a small α parameter ranging from 0.108 to 0.31 suggesting "a great heterogeneity in the distribution of host–pathogen infection probability" (Haas et al., 1999). The dose-responses of the remaining pathogens, *Listeria*, *Yersinia*, Hepatitis E virus, and *Legionella*, were all modeled using animal subjects such as primates, mice, and pigs (2), respectively (Smith et al., 2008; Lathem et al., 2005; Bouwknegt et al., 2009; and Armstrong and Haas, 2007). *Yersinia* was fitted to a Beta-Poisson model, while the others to an exponential model. Animal subjects were selected due to a lack of data on human dose-response for these pathogens. Dose-responses of indicator organisms were not included in this study.

4.1.3. Survival

Survival times of pathogens in biosolids were scarce. Adenovirus and *Listeria* were reported to actually survive in sludge and manure-compost, respectively. Survival of Adenovirus type 4 was based on an average of Adenovirus types 40 and 41 which were stated to persist in secondary sewage effluent at 15°C (Enriquez et al., 1995). *Listeria*, based on a study by Kim et al. (2010), was found to survive for 28 days in manure-compost. Similar to biosolids, *Ascaris* was found to persist in soil for three to four years (Jackson et al., 1977; Griffiths et al., 1978). Survival of other pathogens and indicator

organisms has been extensively studied in various bodies of water. In 1983, Feachem et al. showed that *Entamoeba histolytica* can survive in fresh water at a temperature range of 20-30°C for 15 to 30 days. *Helicobacter* was also found to survive in fresh water at 16-23°C (Adams et al., 2003) and at 25°C in the absence of light (Azevedo et al., 2008). Cook et al. (2007) and Ngazoa et al. (2007) studied the decay and survival of pathogens in river water. It was reported that *Campylobacter* decays in underground river water, whereas Norovirus can survive at 4°C from a viral reduction at 20 and 30 days. Research has shown that *Clostridium perfringens*, *Yersinia*, Astrovirus and Rotavirus survive in groundwater at various temperatures (Filip et al., 1988 and Espinosa et al., 2008). Likewise, it was found that *Shigella* tends to last in wells for approximately 22 days (Henis, 1987). *Cryptosporidium* and *Giardia* have been known to persist in water from sediment experimentations at 23°C (Medema et al., 1998). Koudela et al. (1999) and Ramaiah et al. (2004) studies showed that *Microsporidia* and *Vibrio cholerae* can survive in distilled water at 4°C for two years and 75 days from starvation duration in natural, filtered seawater, respectively. In general, the survival of pathogens at various temperatures has been broadly pursued. Studies indicated that *Salmonella* and enteric viruses persist at 10-25°C (John et al., 2005 and Lyon and Faulkner, 2001). At 0-10°C, Coliphage and Hepatitis A virus persists (John et al., 2005). Literature was not found for the survival of Hepatitis E and its survival was assumed to be the same as Hepatitis A. Similar assumptions were applied to pathogenic *E. coli* O157:H and indicator organism *E. coli* to that of coliform bacteria survival at 3-37°C (John et al., 2005). *Enterococci* have been assumed to be equivalent to fecal streptococcus, and its survival was studied by Keswick et al. (1982). Lastly, it was found that *Toxoplasma* can survive at a higher

temperature range at 30-55°C (86-131°F) compared to the pathogens of interest in this study (Dubey et al., 1998). Decay rates were approximated from the survival characteristics described above and are summarized in Appendix D.

4.2. Point Estimated Relative Risks

4.2.1. Relative Risk Metric Given Occurrence (RRMO)

Much effort was put into the collection of data on the relative risk parameters. However, due to the scarceness of data in each parameter, the relative risk given occurrence could only be computed for six pathogens commonly found in biosolids. Figures 4.1 – 4.3 illustrate the input data for the *RRMO* where the error bars are the UB and LB of the mean. Figure 4.4 displays the results of the *RRMO* with its 90 percentile confidence interval. Natural, distinctive breaks were discovered in the *RRMO* and were used to prioritize the relative risk of infection. The *RRMO* results indicate that Adenovirus, relative to the other pathogens, may potentially present the highest concern given its *RRMO* value of 1.75×10^{2}. However, *Giardia* may also be classified as having a high risk of infection as its *RRMO* value is 2.81. *Cryptosporidium*, *Shigella*, and enteric viruses come in next presenting *RRMO* values of 2.21×10^{-1}, 2.08×10^{-1} and 1.23×10^{-2}, respectively. Salmonella presents a low *RRMO* at 9.54×10^{-6}. Using Microsoft Excel 2010, lognormal, gamma, and Weibull distributions were fitted to the *RRMO* by minimizing the squared differences between the model and input of the *RRMO* function. The Weibull model best fitted the *RRMO* and the alpha and beta parameter are 0.25 and 1.01. The 90 percent confidence interval ranged over seven orders of magnitude with the 5^{th} percentile at 5.60×10^{-6} and the 95^{th} percentile at 9.00×10^{1}. Figure 4.5 displays the cumulative probability distribution of the fitted Weibull model.

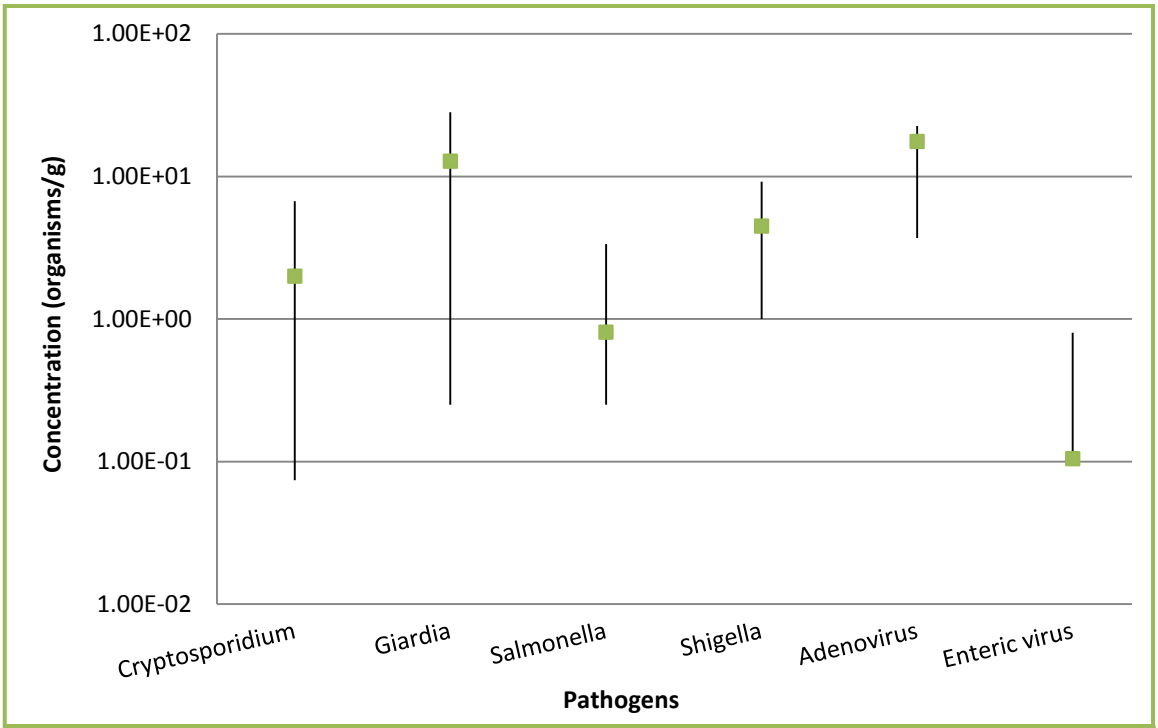

Figure 4.1 **Concentrations of Pathogens in MAD Class B Biosolids**. The error bars indicate the 80 percent confidence interval.

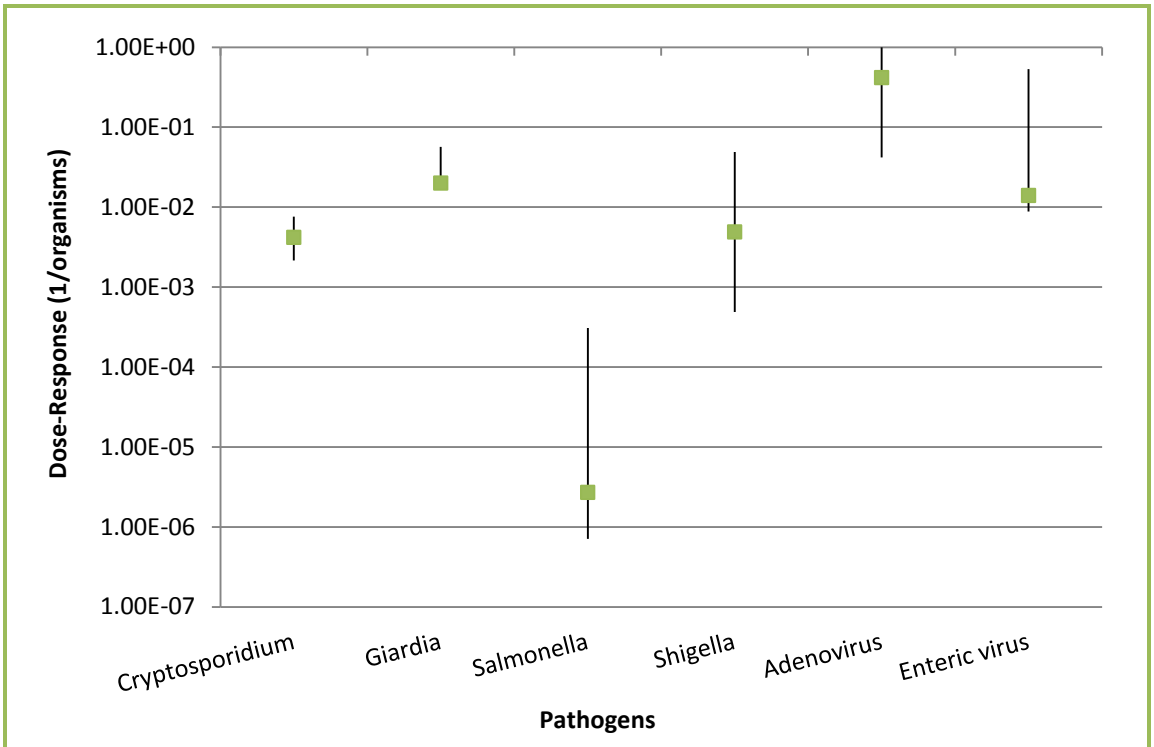

Figure 4.2 **Dose-Responses of Pathogens for which Information on Occurrence in in MAD Class B Biosolids is Available.** The error bars indicate the 80 percent confidence interval unless otherwise denoted by * in which case the error bars indicate the 95 percent confidence interval.

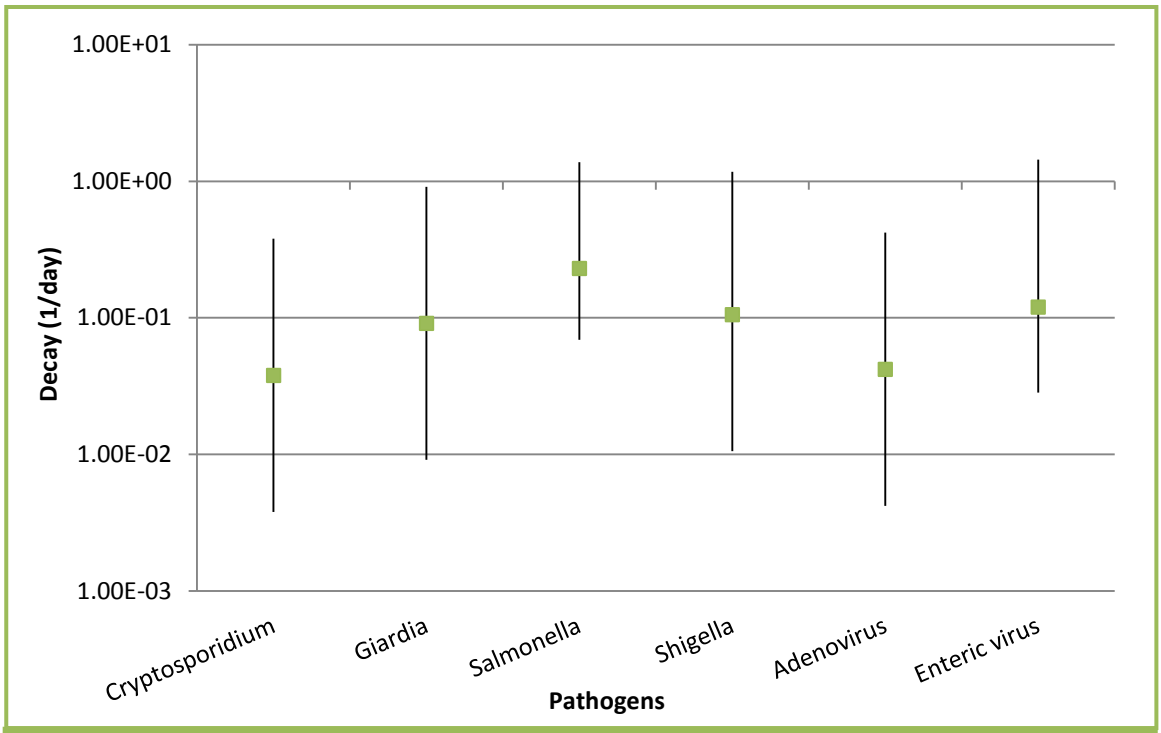

Figure 4.3 **Decay of Pathogens for which Information on Occurrence in MAD Class B Biosolids is Available.** The error bars indicate the 80 percent confidence interval.

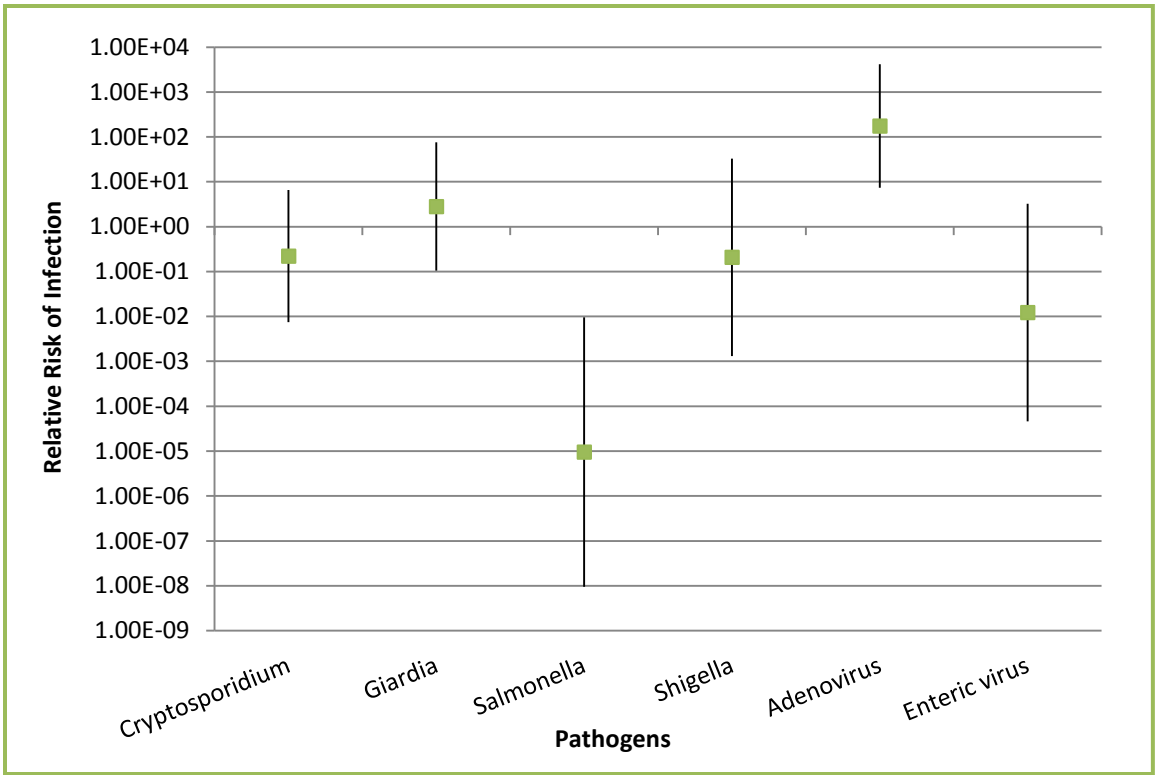

Figure 4.4 RRMO **of Pathogens in MAD Class B Biosolids.** The error bars indicate the 90 percent confidence intervals.

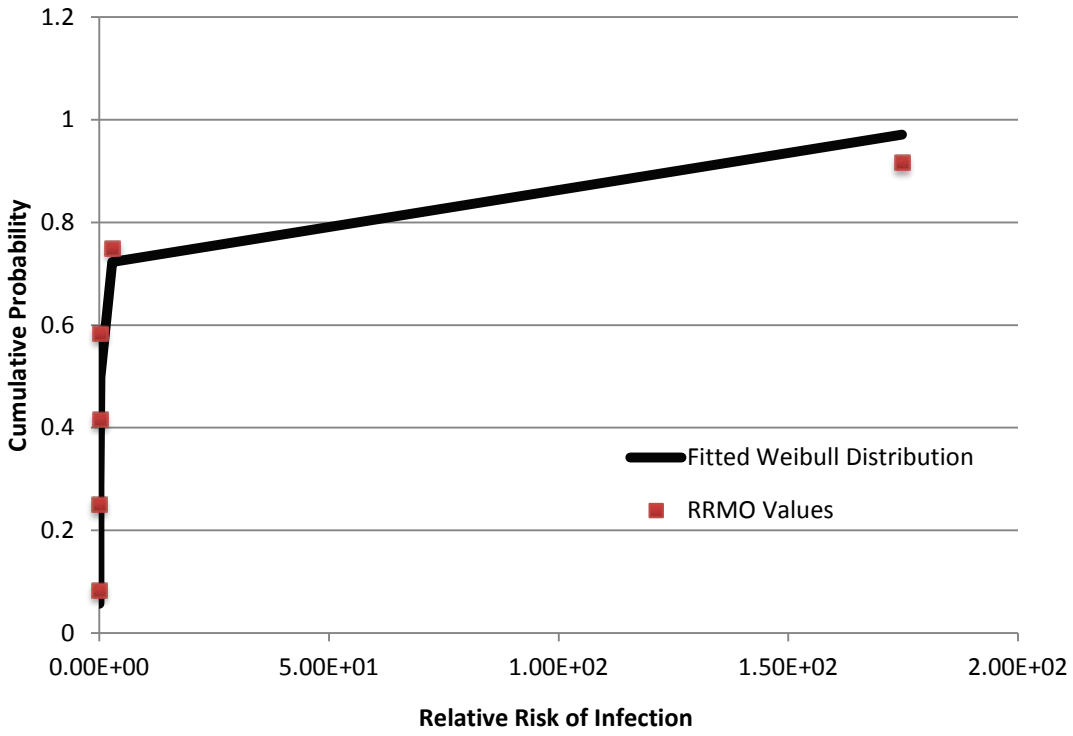

Figure 4.5 **Cumulative Probability Distribution of the Fitted Weibull to the *RRMO*.**

4.2.2. Relative Risk Metric (RRM)

Relative risk of infection was also determined for 17 pathogens based solely on their dose-response and decay characteristics. Figures 4.6 and 4.7 illustrate the *RRM* data inputs where the error bars are the UB and LB of the mean. Figure 4.8 is the generated *RRM* with its 90th Percentile Confidence Intervals. The *RRM* suggests that relative to the other pathogens, *Ascaris* may present the highest risk of infection. Likewise, Adenovirus, Hepatitis A, and Rotavirus have a high risk relative to the other pathogens. These pathogens are classified as high risk because their *RRM* values are 5.16×10^{1}, 2.26×10^{1}, 1.91×10^{1}, and 9.93, respectively. *Cryptosporidium*, *Giardia*, *Campylobacter*,

Shigella, *Vibrio cholerae*, *Yersinia*, Enteric virus, Hepatitis E, and Norovirus pose a medium risk of infection. *E. coli* O157:H, *Listeria*, *Salmonella*, and Astrovirus are deemed as potentially having a low risk of infection. Adenovirus was classified as a high risk pathogen in both the *RRMO* and *RRM*. This may be attributed to the greater concentration and dose-response parameters, and more persistence in the environment as compared to the bacteria, protozoa, and other viruses in this study. In addition, the *RRMO* classified *Giardia* as a high risk of infection, while the *RRM* classified it as medium. This shows the influence of occurrence data on risks.

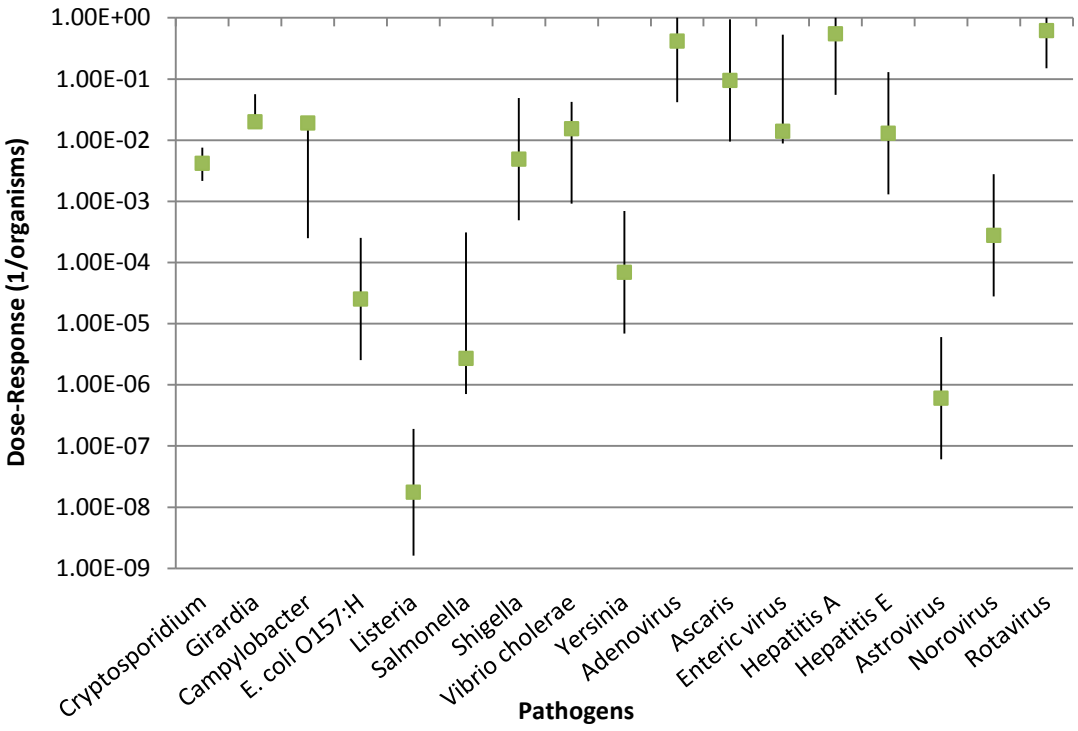

***Figure 4.6* Dose-Responses of Pathogens.** The error bars indicate the 80 percent confidence interval unless otherwise denoted by * in which case the error bars indicate the 95 percent confidence interval.

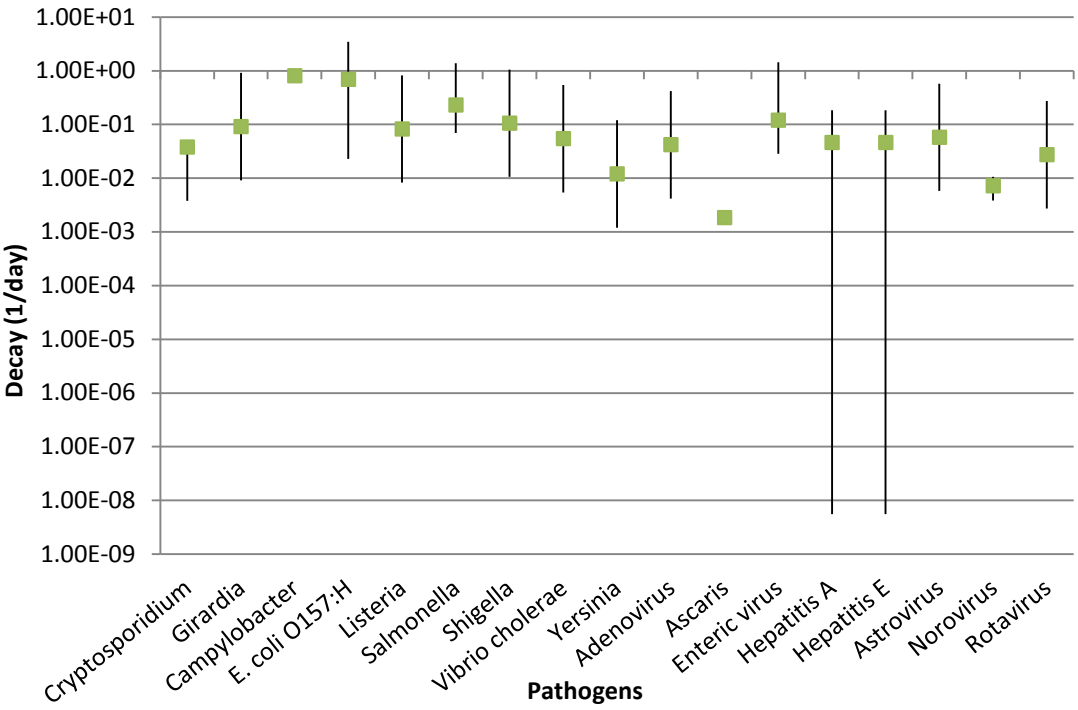

Figure 4.7 **Decay of Pathogens.** The error bars indicate the 80 percent confidence interval unless otherwise denoted by * in which case the error bars indicate the 95 percent confidence interval.

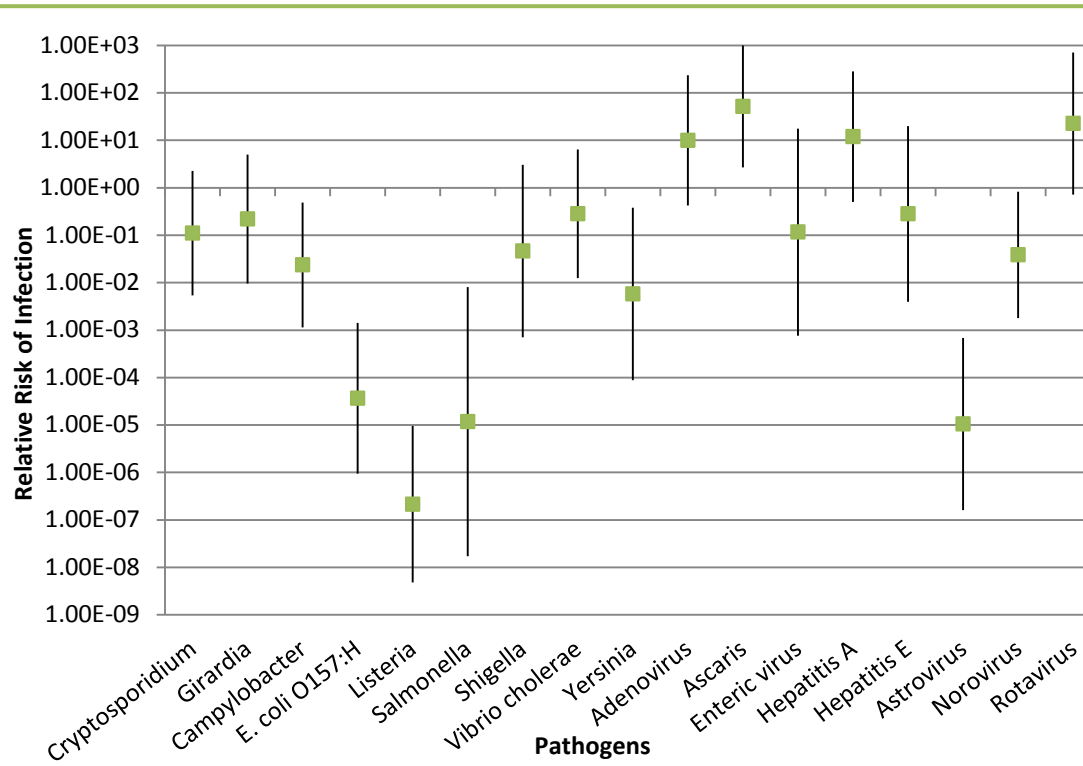

Figure 4.8 RRM **of Pathogens.** The error bars indicate the 90 percent confidence intervals.

Using Microsoft Excel 2010, lognormal, gamma, and Weibull distributions were also fitted to the *RRM* by minimizing the squared difference between the model and input of the *RRM* function. The Weibull model best fitted the *RRM* and the alpha and beta parameter are 0.22 and 0.40. The 90 percent confidence interval ranged over eight orders of magnitude with the 5^{th} percentile at 5.50×10^{-7} and the 95^{th} percentile at 6.00×10^{1}. Figure 4.9 displays the cumulative probability distribution of the *RRM*. Both risk metrics may not present an extremely accurate portray of risks of infection due to a number of uncertainties (i.e. factor of 10, number of samples detected, orders of magnitude variation in the 10^{th} to 90^{th} percentile ranges). The next section discusses which risk parameter contributed the most uncertainty in calculating both the *RRMO* and *RRM*.

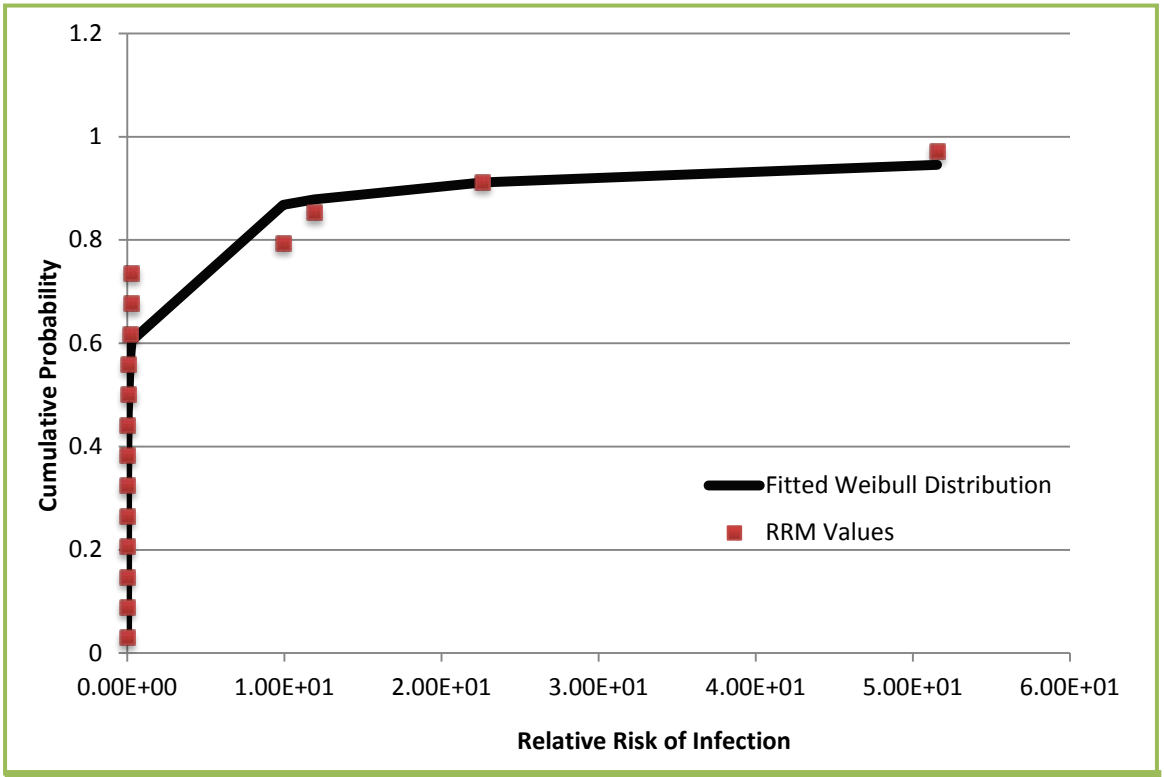

Figure 4.9 **Cumulative Probability Distribution of the Fitted Weibull to the *RRM*.**

4.3. Uncertainty Analysis

4.3.1. RRMO

The variance of each parameter was calculated and presented as percentage of the risk metric to exhibit uncertainty. Results show that *Salmonella had* the most variance in its *RRMO* as compared to *Cryptosporidium*, *Giardia*, *Shigella*, Adenovirus, and enteric viruses (Figure 4.10). The dose-response parameter contributed approximately 77% of uncertainty to the *RRMO* for *Salmonella* (Table 4.1).

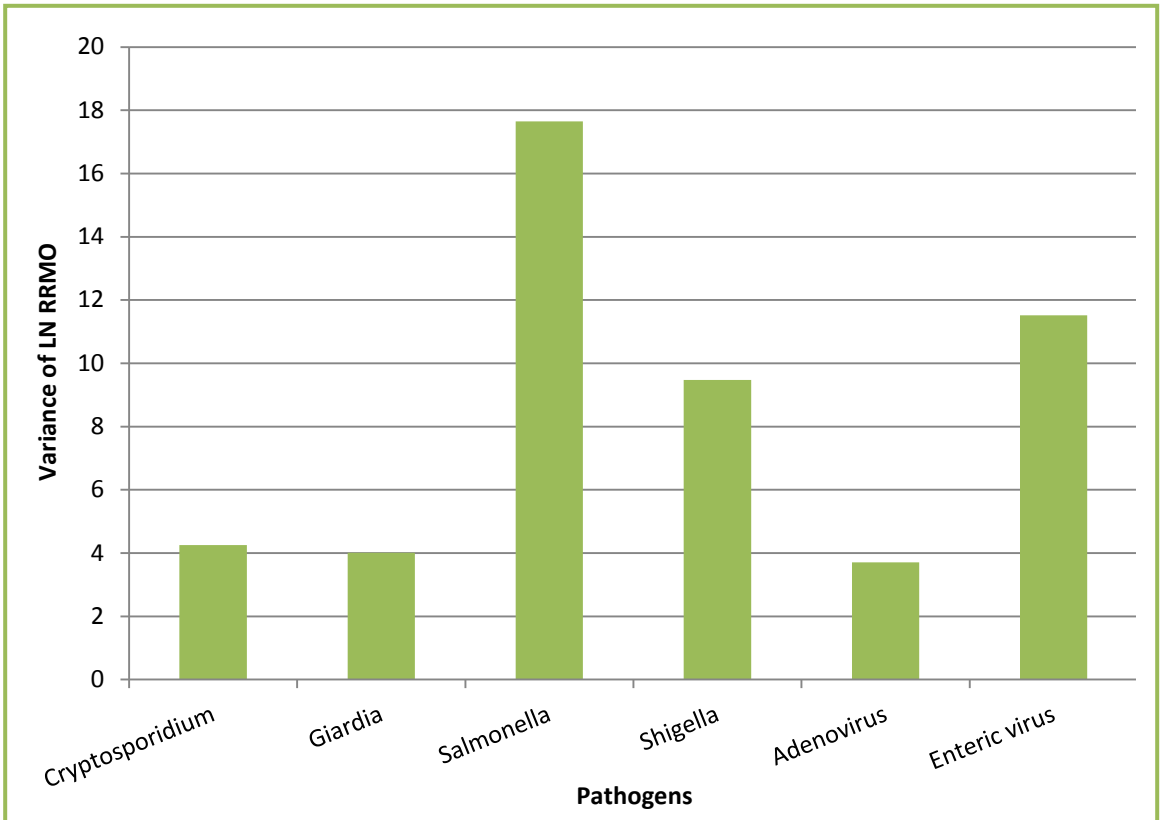

Figure 4.10 **Variance of RRMO in Natural Log-space.**

The high percentage of uncertainty in dose-response for *Salmonella* is due to an uncertainty factor of 114. Dose-response for *Salmonella* ranged from 7.10×10^{-7} to 3.09×10^{-4}, 3 orders of magnitude. Adenovirus, which was classified as having the highest potential of risk of infection, had the lowest uncertainty in the *RRMO*. The contribution of uncertainty was mostly attributed to its decay which had an uncertainty factor of 10. The same reasoning can be applied to the high contributors of uncertainty for *Shigella* and Adenovirus. This implies that more research is need in the area of dose-response and decay. However, for all pathogens in the *RRMO*, occurrence contributed the least amount uncertainty which suggests that data used for these pathogens was of good quality.

Table 4.1 **Percentage of Uncertainty Contribution for RRMO Risk Parameters.**

Pathogen	***Occurrence***	***Dose-Response***	***Decay***
Cryptosporidium	20.95	3.04	**76.00**
Giardia	9.48	9.98	**80.54**
Salmonella	10.70	**77.35**	11.96
Shigella	31.81	**34.10**	**34.10**
Adenovirus	0.25	12.58	**87.17**
Enteric Virus	18.99	**69.98**	11.03

4.3.2. RRM

Figure 4.11 and Table 4.2 show the variance of the *RRM* in natural log-space and the parameter that contributed the most uncertainty, respectively, for each pathogen. Similar to the results of the *RRMO*, *Salmonella* still exhibited the highest variance in relative risk with 86% of its uncertainty contributed by its dose-response parameter. Enteric virus showed the next highest variance with nearly 86% uncertainty from dose-response. Enteric virus dose-response had an uncertainty factor of 38. *Ascaris* displayed the lowest variance in its RRM and almost 100% of its uncertainty was also due to its dose-response parameter.

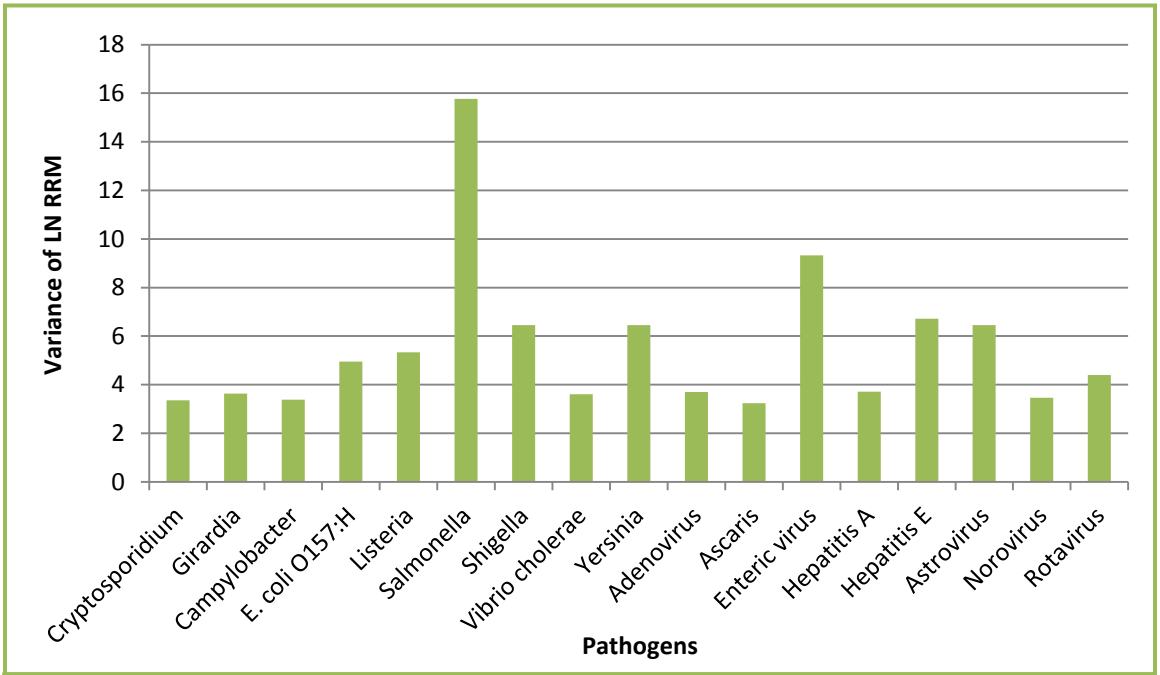

Figure 4.11 **Variance of RRM in Natural Log-space.**

***Table 4.2* Percentage of Uncertainty Contribution for RRM Risk Parameters.**		
Pathogen	***Dose-Response***	***Decay***
Cryptosporidium	3.85	**96.15**
Giardia	11.02	**88.98**
Campylobacter	**77.17**	22.83
E. coli O157:H	**65.22**	34.78
Listeria	39.43	**60.57**
Salmonella	**86.61**	13.39
Shigella	50.00	50.00
Vibrio cholerae	10.38	**89.62**
Yersinia	50.00	50.00
Adenovirus	12.61	**87.39**
Ascaris	**99.55**	0.45
Enteric virus	**86.38**	13.62
Hepatitis A	5.90	**94.10**
Hepatitis E	48.06	**51.94**
Astrovirus	50.00	50.00
Norovirus	**93.06**	6.94
Rotavirus	26.65	**73.35**

Like *Ascaris*, *Cryptosporidium*, *Giardia*, *Campylobacter*, *Vibrio cholerae*, Adenovirus, Hepatitis A and Norovirus had similar variances. For *Campylobacter* and Norovirus, dose-response accounted for 77% and 93%, respectively. However, decay contributed the most uncertainty at 96%, 89%, 90%, 87%, and 94% for *Cryptosporidium*, *Giardia*, *Vibrio cholerae*, Adenovirus, and Hepatitis A, respectively. It was assumed that the decay of Hepatitis E was the same as Hepatitis A; however, the results show that the decay parameter did not have the same effect of uncertainty. Decay only contributed approximately 52% of uncertainty for Hepatitis E, while it contributed 94% for Hepatitis

A. Uncertainty from dose-response was also nearly the same as that of decay for Hepatitis E.

4.4. Conclusions and Further Work

Based on the data current available in the literature, this research sought to prioritize and classify pathogens as high, medium, or low risk infection relative to one another, and determine which parameter contributed the most uncertainty. Results imply that special attention should be focused on *Giardia*, *Ascaris*, Hepatitis A, Rotavirus, but especially Adenovirus, as they may present a high risk of infection if present in MAD Class B Biosolids. Ingestion of protozoa and viruses at infectious doses can lead to gastroenteritis, respiratory illness, heart disease, and paralysis (Metcalf and Eddy, 2003; Haas et al., 1999; and Straub et al., 1993). A number of statistical distributions were evaluated for their ability to fit the calculated values of the metrics for different pathogens. The Weibull model best fitted both the *RRMO* and *RRM* with ranges over seven and eight orders of magnitude, respectively. The large range shows the great variability in the risk that pathogens present. The distribution of the risk metric considers the perspective in which different pathogens are drawn from an overall random distribution of pathogen risks. This may assist in determining the relative risk of infection for pathogens not selected in this study. Results also showed that dose-response and decay parameters contributed the most uncertainty in both the *RRMO* and *RRM* when determining the relative risks of infection from pathogens in MAD Class B Biosolids. There was a lack of information on the distribution of these parameters. It was presumed that these values ranged from a factor of 10 below and above the mean which may have over-estimated the risk of infection. Uncertainty in these parameters is also attributed to

the assumption that pathogens behave similarly to other pathogens in the same class (i.e. decay of Hepatitis E is the same as Hepatitis A). Studies have shown that this is not always the case as organisms vary under different environmental conditions (Sidhu and Toze, 2009; Ahmed and Sorenson, 1997; Straub et al, 1993). Although occurrence was shown to not contribute as considerably as dose-response and decay, sufficient data to include occurrence in the assessment were available for only six pathogens. This study focused on the more general risk parameters that could be applied to any biosolids exposure scenario and does not consist of a detailed QMRA. Consequently, this project gave insight on the prioritization of pathogens that have already been declared "of interest" in the land application of biosolids. An in-depth QMRA, however, was performed by Kumar et al. (2011) which offer site- and scenario- specific results of risk of infection and illness. This study took into consideration high exposure, uptake from different pathways, and the fate and transport of pathogens in land applied biosolids. Kumar et al. (2011) concluded that Adenovirus had the highest risk and *incidental* ingestion of biosolids appeared to be the pathway of most concern. These results confirm the prioritization of this thesis. This study may be utilized as a precursor to a full-blown QMRA to obtain a preliminary consensus of the risk of infection relative to other pathogens in land applied biosolids.

More "accurate methods for recovery, detection, quantification, sensitivity, specificity, virulence, and viability, as well as studies and models addressing transport and fate through the environment" are required to develop exhaustive databases for the occurrence, dose-response and survival exposure assessments (Haas et al., 1999). It is hoped that detection technologies and wastewater and biosolid treatments continue to

advance in order to keep up with the evolution of microorganisms and an increasing population. With constant and effective treatment and management solutions, biosolids may continue to be reused in a manner that protects the public and the environment.

Future work of this research includes collection of more statistical data (occurrence, dose-response, and decay) on pathogens in MAD Class B Biosolids to help reduce uncertainty. Also, statistical analysis should be conducted to determine if there is a significant difference between the *RRMO* and *RRM*, and if so, which metric will give the best estimate of relative risks.

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APPENDIX A: SOLIDS PROCESSING METHODS

Unit operation, unit process, or treatment method	*Function*
Pumping	Transport of sludge and liquid biosolids
Preliminary operations:	
Grinding	Particle size reduction
Screening	Removal of fibrous materials
Degritting	Grit removal
Blending	Homogenization of solids streams
Storage	Flow equalization
Thickening:	
Gravity Thickening	Volume Reduction
Flotation Thickening	Volume Reduction
Centrifugation	Volume Reduction
Gravity-belt Thickening	Volume Reduction
Rotary-drum Thickening	Volume Reduction
Stabilization:	
Alkaline Stabilization	Stabilization
Anaerobic Digestion	Stabilization, mass reduction
Aerobic Digestion	Stabilization, mass reduction
Autothermal Aerobic Digestion (ATAD)	Stabilization, mass reduction
Composting	Stabilization, product recovery
Conditioning:	
Chemical Conditioning	Improve dewaterability
Other Condition Methods	Improve dewaterability
Dewatering:	
Centrifuge	Volume reduction
Belt-filter Press	Volume reduction
Sludge Drying Beds	Volume reduction
Reed Beds	Storage, volume reduction
Lagoons	Storage, volume reduction
Heat Drying:	Weight and volume reduction
Direct Dryers	Weight and volume reduction
Indirect Dryers	
Incineration:	
Multiple-hearth Incineration	Volume reduction, resource recovery
Fluidized-bed Incineration	Volume reduction
Coincineration with solid waste	Volume reduction
Application of biosolids to land:	
Land Application	Beneficial use, disposal
Dedicated Land Disposal	Disposal, land reclamation
Landfilling	Disposal
Conveyance and storage	Solids transport and storage

[a]Metcalf and Eddy, 2003

APPENDIX B: COMPILATION OF OCCURRENCE OF PATHOGENS AND INDICATORS IN MAD CLASS B BIOSOLID†

Pathogen	*Mean*	*Standard Deviation*	*LB*	*UB*	*Reference*
Cryptosporidium[a]	2.00E+00[b]	2.57E+00	7.40E-02	6.70E+00	Guzman et al. (2007)
Giardia [c]	1.28E+01[d]	1.62E+00	<2.50E-01	2.82E+01	Chauret et al. (1999)
Campylobacter[d,e]	<1.00E+00	2.00E-01	<1.00E+00	1.00E+01	Pepper et al. (2010)
Clostridium perfringens[d]	4.16E+07	1.86E+08	3.98E+04	8.53E+08	Pepper et al. (2010)
E. coli O157:H[d,e]	<1.00E+00	2.00E-01	<1.00E+00	1.00E+01	Pepper et al. (2010)
Salmonella[d,f]	8.10E-01	2.60E+00	<2.50E-01	3.35E+00	Pepper et al. (2010)
Shigella[d,f]	4.49E+00	5.37E+01	<1.00E+00	9.20E+00	Pepper et al. (2010)
Adenovirus[d]	1.76E+01	1.33E+01	3.70E+00	2.26E+01	Pepper et al. (2010)
Ascaris[d,e]	<2.50E-01	5.00E-02	<2.50E-01	2.50E+00	Pepper et al. (2010)
Coliphage[d,f]	2.09E+05	3.38E+12	<1.00E+00	1.92E+07	Pepper et al. (2010)
Enteric virus[d,f]	1.05E-01	2.00E-01	<2.50E-01	8.00E-01	Pepper et al. (2010)
Fecal coliforms[d,f]	1.27E+07	3.62E+07	5.17E+01	1.58E+08	Pepper et al. (2010)
E. coli[d]	3.16E+03	2.00E+01	6.05E+00	1.12E+06	Wong et al. (under review)
Enterococci[g]	1.27E+04	3.20E+05	1.00E-20	3.15E+05	Pepper et al. (2010)

†Concentrations are shown as organisms/g where the UB and LB are assumed to be the 80 percent confidence interval.
[a] Viable oocysts.
[b] Geometric mean and standard deviation.
[c] Total cysts.
[d] Arithmetic mean and standard deviation.
[e] All values less than detection.
[f] The MLE method used for mean and standard deviation because some values are below detection.
[g] Utilized values of Fecal streptococcus.

APPENDIX C: COMPILATION OF THE DOSE-RESPONSE OF PATHOGENS IN MAD CLASS B BIOSOLIDS†

Pathogen	*Mean*	*Standard Deviation*	*LB*	*UB*	*Reference*
Cryptosporidium††[a,d]	4.19E-03	1.43E+00	2.15E-03	7.57E-03	Haas et al. (1999); Teunis et al. (1996)
Giardia††[a,d]	2.00E-02	1.88E+00	1.99E-02	5.66E-02	Teunis et al. (1996)
Campylobacter††[b,d]	1.91E-02	1.99E-01	2.49E-04	1.34E-03	Teunis et al. (1996); Medema et al. (1996)
E. coli O157:H[b,d]	2.53E-05	6.03E+00	2.53E-06	2.53E-04	Powell et al. (2000)
Listeria††[a,g]	1.76E-08	4.26E+00	1.62E-09	1.91E-07	Smith et al. (2008)
Salmonella[b,d]	2.71E-06	4.03E+01	7.10E-07	3.09E-04	Teunis et al. (1999); Haas et al. (1999)
Shigella[b,d]	4.90E-03	6.03E+00	4.90E-04	4.90E-02	As cited by Soller et al. (2004)
Vibrio cholerae††[b,d]	1.54E-02	1.84E+00	9.18E-04	4.21E-02	Haas et al. (1999)
Yersinia[b,e]	6.93E-05	6.03E+00	6.93E-06	6.93E-04	Lathem et al. (2005)
Adenovirus type 4[c,d]	4.17E-01	6.03E+00	4.17E-02	1.00E+00	Couch et al. (1966)
Ascaris[b,d]	9.49E-02	6.03E+00	9.49E-03	9.49E-01	Navarro et al. (2009)
Enteric viruses[b,d]	1.40E-02	1.71E+01	8.80E-03	5.32E-01	Haas et al. (1999)
Hepatitis A virus[c,d,h]	5.49E-01	6.03E+00	5.49E-02	1.00E+00	Ward et al. (1958)
Hepatitis E virus[a,f]	1.30E-02	6.03E+00	1.30E-03	1.30E-01	Bouwknegt et al. (2009)
Astrovirus[c,d]	6.06E-07	6.03E+00	6.06E-08	6.06E-06	Haas et al. (1999)
Legionella [a,f]	6.00E-02	6.03E+00	6.00E-03	6.00E-01	Armstrong and Haas (2007)
Norovirus[a,d]	2.78E-04	6.03E+00	2.78E-05	2.78E-03	Teunis et al. (2008)
Rotavirus[b,d]	6.19E-01	2.95E+00	1.50E-01	1.00E+00	Ward et al. (1986); Regli et al. (1991); Haas et al. (1993)

† UB and LB were assumed to be the 80 percent confidence interval.
††UB and LB were reported as the 95 percent confidence interval.
[a] Exponential dose-response.
[b] Exponential dose-response estimated by Beta-Poisson parameters.
[c] Exponential dose-response estimated by exponential decay.
[d] Based on human subject.
[e] Based on mice subjects.
[f] Based on pig subjects.
[g] Based on primate subjects.
[h] Dose in grams of feces of excreting infected individuals.

APPENDIX D: COMPILATION OF THE DECAY OF PATHOGENS AND INDICATORS IN MAD CLASS B BIOSOLIDS†

Pathogen	*Mean*	*Standard Deviation*	*LB*	*UB*	*Reference*
Cryptosporidium	3.79E-02	3.98E+00	3.79E-03	3.79E-01	Medema (1998)
Entamoeba histolytica	1.15E-01	1.75E+01	7.68E-02	1.54E-01	Feachem (1983)
Giardia	9.12E-02	3.98E+00	9.12E-03	9.12E-01	Medema (1998)
Microsporidia	3.14E-03	3.98E+00	3.14E-04	3.14E-02	Koudela (1999)
Campylobacter *	8.06E-01	6.22E-02	7.44E-01	8.69E-01	Cook (2007)
Clostridium perfringens	6.00E-03	3.98E+00	6.00E-04	6.00E-02	Filip (1988)
E. coli O157:H	6.91E-01	9.22E-01	2.30E-02	3.46E+00	John (2005)
Helicobacter	5.88E+00	2.57E+00	3.36E-01	1.10E+01	Adams (2003); Azevedo (2008)
Listeria	8.23E-02	3.98E+00	8.23E-03	8.23E-01	Kim (2010)
Salmonella	2.30E-01	4.58E-01	6.91E-02	1.38E+00	John (2005)
Shigella	1.06E-01	3.98E+00	1.06E-02	1.06E+00	Henis (1987)
Vibrio cholerae	5.45E-02	3.98E+00	5.45E-03	5.45E-01	Ramaiah (2005)
Yersinia	1.20E-02	3.98E+00	1.20E-03	1.20E-01	Filip (1988)
Adenovirus type 4	4.20E-02	3.98E+00	4.20E-03	4.20E-01	Enriquez (1995)
Ascaris	1.84E-03	2.13E+01	1.58E-03	2.10E-03	Jackson (1977); Griffiths (1978)
Coliphage	6.91E-02	6.91E-02	5.52E-09	1.84E-01	John (2005)
Enteric viruses	1.20E-01	7.78E+00	2.83E-02	1.44E+00	Lyon and Faulkner (2001)
Hepatitis A virus	4.61E-02	9.22E-02	5.52E-09	1.84E-01	John (2005)
Hepatitis E virus	4.61E-02	9.22E-02	5.52E-09	1.84E-01	John (2005)
Astrovirus	5.76E-02	3.98E+00	5.76E-03	5.76E-01	Espinosa (2008)
Norovirus	7.20E-03	1.47E+01	3.84E-03	1.05E-02	Ngazoa (2007)
Rotavirus	2.74E-02	3.98E+00	2.74E-03	2.74E-01	Espinosa (2008)
Toxoplasma	2.10E-01	3.84E+01	3.60E-02	3.84E-01	Dubey (1998)
Fecal coliforms	4.51E-01	3.98E+00	4.51E-02	4.51E+00	McFeters (1974)
E. coli	6.91E-01	9.22E-01	2.30E-02	3.46E+00	John (2005)
Enterococci	5.28E-01	3.98E+00	5.28E-02	5.28E+00	Keswick (1982)

† Units are presented as 1/day where the UB and LB were assumed to be the 90 percent confidence interval unless otherwise denoted by * in which case the UB and LB indicate the 95 percent confidence interval.